Kewal K. Jain

# Textbook of Personalized Medicine

*Second Edition*

Humana Press

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Jain PharmaBiotech, Basel, Switzerland

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*"Tonight, I'm launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes — and to give all of us access to the personalized information we need to keep ourselves and our families healthier."*

*—President Barack Obama,*
*State of the Union Address,*
*20 January 2015, USA*

# Preface to the Second Edition

Considerable advances have taken place in technologies used for advancing personalized medicine, and it is increasingly applied in clinical use. This has required expansion and revision of some parts of the first edition published in 2009. The style has been maintained due to the positive feedback of readers, including scientists, pharmacists, physicians, and lay persons interested in this topic. The book provides a concise and comprehensive source of reference for those involved in healthcare management, planning, and politics. As a single author book, it avoids the overlaps and missing areas frequently found in multiauthor books. Moreover, the time to publication is reduced by avoiding the long delays of numerous authors who do not keep the deadlines.

The book includes 700 references selected from thousands of publications during the past decade and appended at the end of each chapter. Some of the references included were prepublication versions at the end of 2014 that will not be formally published until 2015. The text is supplemented by 31 illustrations and 56 tables. Algorithms are included as a guide to those involved in the management of important diseases where decision making is involved due to the multiple choices available.

Finally, I thank the editorial staff of Springer, particularly Patrick Marton and David Casey, for their help and encouragement in this project.

Basel, Switzerland — Kewal K. Jain

# Preface to the First Edition

Personalized medicine, which simply means selection of treatment best suited for an individual, involves integration and translation of several new technologies in clinical care of patients. The scope is much broader than indicated by the term genomic medicine, because many non-genomic factors are taken into consideration in developing personalized medicine. Basic technologies for personalized medicine, of which molecular diagnostics has the biggest share, are mentioned briefly and appropriate references are given for further information. Commercial aspects are discussed briefly in a chapter and detailed analysis of markets and companies involved in personalized medicine is presented in a special report on this topic. There is increasing interest in personalized medicine. Considerable advances have taken place in molecular biology and biotechnology to make personalized medicine a viable option, but some misconceptions still exist, both in the academic and in the commercial sectors. There is lack of a suitable source of information that provides both the fundamentals as well as applications of personalized medicine. As the latest version of the first monograph on personalized medicine published in 1998, this volume, *Textbook of Personalized Medicine*, summarizes the author's efforts during the past decade as well as reviews of selected studies done during this period in a readable format for physicians and scientists. It is hoped that physicians, pharmacists, scientists, and interested lay readers with basic scientific knowledge will find this book useful.

Basel, Switzerland — Kewal K. Jain

# About the Author

**Professor K. K. Jain** is a neurologist/neurosurgeon by training and has been working in the biotechnology/biopharmaceuticals industry for several years. He received graduate training in both Europe and USA, has held academic positions in several countries, and is a Fellow of the Faculty of Pharmaceutical Medicine of the Royal College of Physicians of UK. Currently, he is a consultant at Jain PharmaBiotech. He has been working on developing personalized therapy by integrating new technologies in addition to genomics since 1997. His monograph with the title *Personalized Medicine* published in 1998 was the first treatise on this topic. Over the years, it went through several editions until it evolved into the *Textbook of Personalized Medicine*, published by Springer in 2009. It was translated into Japanese in 2012 and the current version is the second edition of this book.

Professor Jain's 452 publications include 25 books (5 as editor + 20 as author) and 50 special reports, which have covered important areas in biotechnology, gene therapy, and biopharmaceuticals. His important recent books include *Handbook of Nanomedicine* (Springer 2008; Chinese edition, Peking University Press 2011; 2nd ed Springer 2012), *Handbook of Biomarkers* (Springer 2010), *Handbook of Neuroprotection* (Springer 2010), *Drug-induced Neurological Disorders*, 3rd ed (Hogrefe 2011), *Applications of Biotechnology in Cardiovascular Disorders* (Springer 2011), *Applications of Biotechnology in Neurology* (Springer 2013), and *Applications of Biotechnology in Oncology* (Springer 2014). He has edited *Applied Neurogenomics* (January 2015).

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# Abbreviations

| | |
|---|---|
| ACE | Angiotensin-converting enzyme |
| ADME | Adsorption, distribution, metabolism, excretion |
| ADR | Adverse drug reaction |
| CE | Capillary electrophoresis |
| CF | Cystic fibrosis |
| CML | Chronic myeloid leukemia |
| CT | Computerized tomography |
| CRADA | Cooperative Research & Development Agreement |
| CYP | Cytochrome P |
| DARPA | Defense Advanced Research Projects Agency |
| DHPLC | Denaturing high performance liquid chromatography |
| DNA | Deoxyribonucleic acid |
| DR | Dopamine receptor |
| dsDNA | Double-stranded DNA |
| eNOS | Endothelial nitric oxide synthase |
| EPOE | Apolipoprotein E |
| HER | Electronic health records |
| FDA | Food and Drug Administration (USA) |
| FISH | Fluorescent in situ hybridization |
| GFP | Green fluorescent protein |
| HCV | Hepatitis C virus |
| HER-2 | Human epidermal growth factor receptor-2 |
| HIV | Human immunodeficiency virus |
| IL | Interleukin |
| JAK | Janus kinase |
| MAb | Monoclonal antibody |
| MALDI-TOF | Matrix Assisted Laser Desorption Ionization Time of Flight |
| MDR | Multidrug resistance protein |
| MHC | Major histocompatibility complex |
| MRI | Magnetic resonance imaging |
| mRNA | Messenger RNA |

| | |
|---|---|
| MS | Mass spectrometry |
| mtDNA | Mitochondrial DNA |
| MTHFR | Methylenetetrahydrofolate reductase |
| NCI | National Cancer Institute |
| NGS | Next generation sequencing |
| PARP | Poly (ADP-ribose) polymerase |
| PCR | Polymerase chain reaction |
| PET | Positron emission tomography |
| PNA | Peptide nucleic acid |
| POC | Point-of-care |
| RCAT | Rolling circle amplification technology |
| RFLP | Restriction fragment length polymorphism |
| RNA | Ribonucleic acid |
| SBIR | Small Business Innovation Research |
| SELDI | Surface-enhanced laser desorption/ionization |
| SNP | Single nucleotide polymorphism |
| STAT | Signal transducer and activator of transcription |
| TDM | Therapeutic drug monitoring |
| TKI | Tyrosine kinase inhibitor |
| TNF | Tumor necrosis factor |
| TPMT | Thiopurine methyltransferase |
| WGA | Whole genome association |
| WGS | Whole genome sequence |
| ZFP | Zinc finger proteins |

# Chapter 1
# Basic Aspects

Most of the current drugs are approved and developed based on their performance in a large population of people but medicine of the future is developing as personalized solutions for a particular patient's needs. In case of complex disorders, the conventional "one-drug-fits-all" approach involves trial and error before an appropriate treatment is found. Clinical trial data for a new drug merely shows the average response of a study group. There is, however, considerable individual variation; some patients show no response whereas others show a dramatic response. It is obvious that the concept "one medicine for all patients with the same disease" does not hold and a more individualized approach is needed. Although individualization of certain treatments has been carried out in the pregenomic era, the concept of personalized medicine as described in this report follows progress in study of human diseases at molecular level, advances in molecular diagnostics and drug development based on genomics, proteomics, metabolomics and biomarkers. The aim of the personalized medicine is to match the right drug to the right patient and in some cases, even to design the treatment for a patient according to genotype as well as other individual characteristics. A broader term is integrated healthcare, which includes development of genomics-based personalized medicine, predisposition testing, preventive medicine, combination of diagnostics with therapeutics and monitoring of therapy. This fits in with the concept of system biology as applied to healthcare and termed systems medicine.

## Definition of Personalized Medicine

There is no officially recognized definition of personalized medicine. The term "personalized medicine" was first used as the title of a monograph in 1998 (Jain 1998) and started to appear in MEDLINE in 1999 but most of the literature relevant to personalized medicine is still indexed under pharmacogenomics and pharmacogenetics (Jain 2002). Various terms that are used to describe the concept of personalized medicine are listed in Table 1.1.

K.K. Jain, *Textbook of Personalized Medicine*, DOI 10.1007/978-1-4939-2553-7_1,

**Table 1.1** Selected terms relevant to the concept of personalized medicine

| |
|---|
| Customized drug therapy |
| Genomic medicine |
| Genotype-based therapy |
| Individualized medicine or individual-based therapy |
| Information-based medicine |
| Integrated healthcare |
| Omics-based medicine: pharmacogenomics/pharmacogenetics/pharmacoproteomics/ pharmacometabolomics |
| Precision medicine |
| Rational drug selection |
| Stratified medicine |
| Systems medicine |
| Tailored therapy |
| Translational medicine |



The term "genomic medicine" implies that the sequencing of the human genome has enabled the practice of medicine to enter an era in which the individual patient's genome will help determine the optimal approach to care, whether it is preventive, diagnostic, or therapeutic. Genomic medicine is an inadequate description because personalized medicine was there before the genome was sequenced and other 'omics' besides genomics play a role. Stratified medicine is recognized as a key strategic approach to the diagnosis as well as treatment of disease and depends critically upon information; the integration of existing data sets to form a comprehensive 'personal' healthcare record and the generation of new data describing patient characteristics – genotype and phenotype – to permit 'stratification.'

Personalized medicine, also referred to as individualized medicine, simply means the prescription of specific treatments and therapeutics best suited for an individual taking into consideration both genetic and environmental factors that influence response to therapy. Genomic/proteomic technologies have facilitated the development of personalized medicines but other technologies such as metabolomics are also contributing to this effort. Personalized medicine is the best way to integrate new biotechnologies into medicine for improving the understanding of pathomechanism of diseases and management of patients.

This process of personalization starts at the development stage of a medicine and is based on pharmacogenomics and pharmacogenetics, which will be discussed in detail in later chapters. The concept of personalized medicine will enable pharmaceutical companies to develop more effective medicines with fewer side effects. Physicians will have access to genetic profiles of their patients that will allow them to use existing medicines more effectively and safely, and individuals will be able to better manage their health based on an understanding of their genetic profile. In contrast to trial and error approach of some conventional therapies, personalized medicines aim to achieve a better match of drugs to patients so that the right

treatments are given to the right patients at the right time. Personalized medicine is become a reality with the sequencing of the human genome, advances in medical genetics and several technologies including medical diagnostics, single nucleotide polymorphism (SNP) genotyping and proteomics.

Some consider the word "personalized" to be somewhat indicative of exclusivity and prefer to use the term integrated healthcare to indicate the integration of diagnostics, screening, prevention, therapy and treatment monitoring as the future trend in medicine. The problem with the term "integrated healthcare" is that it is already being used to indicate the integration of classical medicine with alternative medicine. Integration of diagnosis and treatment is implied in the development of personalized medicine and the author of this report prefers to use the term "personalized medicine" for the system and to refer to the individual drugs as personalized medicines. Systems medicine also implies integration of various disciplines. The term "precision medicine" is used because diagnostic, prognostic, and therapeutic strategies are precisely tailored to each patient's requirements (Mirnezami et al. 2012).

## History of Medical Concepts Relevant to Personalized Medicine

A general overview of the development of concepts in patient management will provide a background for the development of personalized medicine and various landmarks are shown in Table 1.2.

According to the Ayurveda, a human being is a model of the universe where the basic matter and the dynamic forces (Dosha) of the nature determine health and disease, and the medicinal value of any substance (plant and mineral). The Ayurvedic practices (mainly diet, life style, and meditation) aim to maintain the Dosha equilibrium (Chopra and Doiphode 2002). Despite a holistic approach aimed to cure disease, therapy is customized to the individual's constitution (Prakruti) – ancient counterpart of genotype.

The traditional Chinese medicine with acupuncture and herbs takes individual variations into consideration and this system is still practiced in new China (Jain 1973). Sasang typology, a Korean traditional medical system, explains the individual differences in behavioral patterns, physical characteristics and susceptibility to a certain disease based on their biopsychological traits (Chae et al. 2004). It is a sort of personalized medicine that includes guideline for safe and effective use of acupuncture and medical herbs, particularly those with significant adverse events, such as Ma-Huang (Ephedra Sinica) and Aconite. It is also to be noted that many of the ancient systems of healthcare survive in the form of so-called "alternative therapies" and most of the population of present day world still relies on these treatment. There is a personal touch or individualization in many of these treatments for lack of any standard or universal therapies. The healer has a feel for each individual patient and the treatment is modified according to the needs and personality of the patient.

**Table 1.2** Landmarks in the historical development of personalized medicine

| Era/year | Medical system/concept |
|---|---|
| 10,000 years ago | Primitive medicine a mixture of magic, rituals and potions and personal touch |
| 6000–3000 BC | Mesopotamian and Egyptian medicine: Rituals plus medicines from natural sources, some of which are still in use and some are the basis of currently used medicines |
| 4000–500 BC | Ayurveda, the ancient medical system of India with a blend of transcendental meditation and herbs, provided the first concept of individualized healthcare |
| 3000 BC | Ancient Chinese medicine used herbs and acupuncture, which are still in use |
| 510 BC | The Greek Pythagoras observed that only some individuals (now known to have deficiency of G6PD) developed a potentially fatal reaction after ingesting fava beans |
| 500 BC – 500 AD | Greek medicine separated from rituals and religion. Clinical observations on diseases but few medicines |
| 500 AD – 1500 AD | Medieval period of medicine. Further development of Greek tradition in Arabic medicine. Start of hospitals and universities |
| Sixteenth to eighteenth centuries | Important discoveries in anatomy and physiology but no pharmacological advances in middle ages. Patient care was personalized for lack of standard treatments |
| 1789 | Founding of homeopathy based on "like cures like" by Samuel Hahnemann in Germany. Homoeopathic prescribing is highly individualized to a person's "constitutional picture" rather than to specific diseases |
| Nineteenth century, late | Start of modern medicine. Claude Bernard's (1813–1878) introduction of the scientific method into medicine, founded on observation and proved by experiments, started to endanger personal aspects of treatment |
| Twentieth century | Most of the advances in medicine were made in this century including imaging techniques, laboratory diagnostics and modern surgical techniques. Important advances in later decades include discovery of biotechnology-based products, molecular diagnostics, genomics, proteomics, biochips, antisense therapy and gene therapy |
| Twentieth century second half | Introduction of randomized, double-blind clinical trials was inconsistent with the individualized treatment as it leveled out variations of individual responses to treatment |
| 1908 | Introduction of the word 'gene' into the German language as 'Gen' by Wilhelm Johannsen and subsequent terms "genotype" and "phenotype" |
| 1920–1950 | Scientific basis of pharmacology developed with concept of receptors |
| 1931 | Publication of a book suggesting that individual differences in responses to drugs should be anticipated because of the marked individual differences in each person's genetic constitution (Garrod 1931) |
| 1953 | Identification of the double-stranded structure of the DNA (Watson and Crick 1953) |
| 1955 | Observation of a high incidence of hemolysis on exposure to antimalarial drugs among individuals with glucose-6-phosphate dehydrogenase deficiency (Beutler et al. 1955) |
| 1956–1957 | Concept of pharmacogenetics: recognition that adverse reactions to drugs can be caused by genetically determined variations in enzyme activity (Kalow 1956; Motulsky 1957) |

(continued)

**Table 1.2** (continued)

| Era/year | Medical system/concept |
|---|---|
| 1959 | Definition of the special field of pharmacogenetics combining the techniques of pharmacology and genetics (Vogel 1959) |
| 1962 | Publication of the first monograph on pharmacogenetics (Kalow 1962) |
| 1968 | Development of principles of population screening, which later formed the basis of application of genetics for population screening (Wilson and Jungner 1968) |
| 1980–1990 | Further developments in scientific pharmacology. Characterization of receptors by ligand-binding studies. Start of impact of molecular biology on pharmacology |
| 1985 | Discovery of polymerase chain reaction (Mullis et al. 1986) |
| 1986 | Coining of the word "Genomics" by Roderick as title of the journal, which started publication in 1987 (Kuska 1998) |
| 1990–2000 | The genomic decade. Sequencing of the human genome. Parallel miniaturization in robotics and computer systems. Application of genomic technologies to drug development: pharmacogenomics. Cell and gene therapies |
| 1993 | Concept of using molecular nanotechnology to base medical therapy on the biochemical individuality of specific patients (Fahy 1993) |
| 1995 | Coining of the term "proteomics" (Wilkins et al. 1995) |
| 1997 | The term "pharmacogenomics" appears in the literature (Marshall 1997) |
| 1998 | First monograph with the title "Personalized Medicine and Pharmacogenetics (Jain 1998), published by Decision Resources Inc. |
| 2000 | Sequencing of the human genome completed |
| 2001–2010 | Post-genomic decade. Impact of genomics combined with proteomics in drug discovery and development. Development of personalized medicine and integration of diagnosis with therapy in healthcare |
| 2008 | Genetic Information Nondiscrimination Act passed in the US |



It is obvious that the progress made during the past few decades surpasses that made in the whole of previous medical history. Modern medicine is considered to start in the nineteenth century although several important discoveries, notably smallpox vaccine were made close to the end of the eighteenth century. Modern pharmaceuticals and drug discovery started to develop in the twentieth century with most of the advances taking place in the second half and the most important ones in the last decade.

The role of physicians in making necessary judgments about the medicines that they prescribe has often been referred to as an art, reflecting the lack of objective data available to make decisions that are tailored to individual patients. Now we are on the verge of being able to identify inherited differences between individuals, which can predict each patient's response to a medicine. Review of history of medicine shows that development of personalized medicine will be an evolution and not revolution in medicine. Medicine has always been evolving and will continue to evolve although the progress may appear slow at times. Some remarkable discoveries such as the double helix of DNA and polymerase chain reaction did not have an immediate impact on practice of medicine.

## Molecular Biological Basis of Personalized Medicine

Although several factors are involved in the development of personalized medicine, developments in molecular biology have played an important role. Some basic terms are defined briefly in this section.

### *The Human Genome*

The total genetic material of an organism, that is, an organism's complete DNA sequence is called a genome. The human genome is extremely complex, and the estimated number of genes has varied considerably during the past years. GENCODE 19 contained 20,719 protein-coding genes. A study has mapped peptides detected in seven large-scale proteomics studies to ~60 % of the protein-coding genes in the GENCODE annotation of the human genome (Ezkurdia et al. 2014). The investigators described a set of 2,001 potential non-coding genes based on features such as weak conservation, a lack of protein features, or ambiguous annotations from major databases. Peptides were identified for only 3 % of these genes. Most of these genes behave like non-coding genes rather than protein-coding genes and are unlikely to code for proteins under normal circumstances. If one excludes them from the human protein-coding gene catalog, the total number of genes in the human genome is reduced to ~19,000.

#### ENCODE

Following sequencing of the human genome in 2001, the aim of ENCODE (ENCyclopedia Of DNA Elements) became description of all functional elements encoded in the human genome (nature.com/encode). The federal project involved 440 scientists from 32 laboratories around the world. Nine years after launch, its main efforts culminate in the publication of 30 coordinated papers (Skipper et al. 2012). Collectively, the papers describe 1,640 data sets generated across 147 different cell types. Among the many important results there is one that stands out above them all: more than 80 % of the human genome's components have now been assigned at least one biochemical function. ENCODE findings have implications for many fields in biology. Some key findings are:

The human genome has at least four million gene switches that reside in bits of DNA that once were dismissed as "junk DNA" but that turns out to play critical roles in controlling how cells, organs and other tissues behave. At least 80 % of this DNA is needed for normal function and is active. Human DNA is a very long strand – 3 m of DNA stuffed into a microscopic nucleus of a cell – that it fits only because it is tightly wound and coiled around itself and is referred to as a "hairball with a 3D structure. In the past, when only the uncoiled length of DNA was analyzed,

the controlling regions appeared to be far from the genes they affect. Now ENCODE researchers have discovered that small segments of junk DNA are often quite close to genes they control.

The result of the ENCODE studies is an annotated road map of much of this DNA. The system of switches select the genes used in a cell as well as determine when they are used and their fate, e.g. whether a cell becomes a liver cell or a neuron. Many complex diseases appear to be caused by tiny changes in hundreds of gene switches. These findings will have immediate applications for understanding how alterations in the non-gene parts of DNA contribute to human diseases, which may in turn lead to new treatments. They can also help explain how the environment can affect disease risk. In the case of identical twins, small changes in environmental exposure can slightly alter gene switches, with the result that one twin gets a disease and the other does not.

Gene switches are linked to a range of human diseases: multiple sclerosis, lupus, rheumatoid arthritis, Crohn's disease, celiac disease and even to traits like height. The discoveries also reveal the genetic changes that are important in cancer and why. Most of the thousands of DNA changes in cancer cells were found not in genes, but in the so called "junk DNA". The challenge is to figure out which of those changes are driving the cancer's growth, which has implications for the management of cancer. In prostate cancer, e.g. there are mutations in important genes that do not readily respond to drugs. ENCODE, by showing which regions of the "junk DNA" control those genes, provides another approach to attack them by targeting the controlling switches.

## *Chromosomes*

Each human chromosome is a long linear double-stranded DNA molecule (except the mitochondrial chromosome) ranging in size from 50 to 250 million base pairs. An average chromosome contains 2,000–5,000 genes within 130 million base pairs and is equal to about 130 cM of genetic material. A typical microband on a chromosome contains 3–5 million bp and 60–120 genes. There are approximately 400 million nucleotides in a human chromosome, but only about 10 % of them actually code for genes; the rest may play different roles such as regulating gene expression.

The complex of DNA and proteins of a chromosome is called chromatin and consists of histones and non-histone proteins. The basic structural unit of chromatin is a nucleosome – a complex of DNA with a core of histones. The amount of DNA associated with each nucleosome is about 200 base pairs. Nucleosomes are further compacted to solenoids which are packed into loops and each of these contains about 100,000 base pairs of DNA. The loops are the fundamental units of DNA replication and/or gene transcription. A karyotype describes an individual's chromosome constitution. Each of the 46 human chromosomes can now be counted and characterized by banding techniques.

Chromosomes X and Y are the sex chromosomes. Each man carries an X chromosome and a Y chromosome. Every woman carries two X chromosomes. As there are actually few genes on the Y chromosome, men and women each have one active X chromosome that codes most of the information. More than 99 % of the euchromatic sequence of the X chromosome has been determined. A disproportionately high number of mendelian diseases are documented for the X chromosome; of >1,000 genes, 168 mutations in 113 X-linked genes have been explained, which in many cases were characterized with the aid of the DNA sequence. Examples are defects in the gene responsible for Duchenne muscular dystrophy to and fragile X mental retardation. As men have only one copy of the X chromosome, it is easier to find mutated genes on that one piece of DNA.

## *Genes*

A gene is a sequence of chromosomal DNA that is required for the production of a functional product: a polypeptide or a functional RNA molecule. Genes range in size from small (1.5 kb for globin gene) to large (approximately 2,000 kb for Duchenne muscular dystrophy gene). A gene includes not only the actual coding sequences but also adjacent nucleotide sequences required for the proper expression of genes – that is, for the production of a normal mRNA molecule. Mature mRNA is about one-tenth the size of the gene from which it is transcribed. The same DNA strand of a gene is always translated into mRNA so that only one kind of mRNA is made for each gene. Transcription is gene in action. Genes are often described as blueprints of life and transmit inherited traits from one generation to another.

### The Genetic Code

The sequence of nucleotide bases of the "genetic code" in a particular gene is reflected in the specific sequence of amino acids in the polypeptide produced through the protein synthesis mechanism. The co-linearity between the DNA molecule and the protein sequence is achieved by means of the genetic code. At any position there are four possibilities (A, T, C, and G). Thus, for three bases, there are 43 or 64 possible triplet combinations. These 64 codons constitute the genetic code.

### Gene Expression

The activity of a gene, so called gene "expression" means that its DNA is used as a blueprint to produce a specific protein. Only a small number of these genes, about 15,000, are expressed in a typical human cell, but the expressed genes vary from one

cell to another. Gene expression can be detected by various techniques described in Chap. 2. The discovery that eukaryotic genes are not contiguous sequences of DNA but consist of coding sequences (exons) interrupted by intervening sequences (introns) led to a more complex view of gene expression. The temporal, developmental, typographical, histological and physiological patterns in which a gene is expressed provide clues to its biological role. Malfunctioning of genes is involved in most diseases, not only inherited ones.

All functions of cells, tissues and organs are controlled by differential gene expression. As an example, red blood cells contain large amounts of the hemoglobin protein that is responsible for carrying oxygen throughout the body. The abundance of hemoglobin in red blood cells reflects the fact that its encoding gene, the hemoglobin gene, is actively transcribed in the precursor cells that eventually produce red blood cells. In all other cells of the body, the hemoglobin gene is silent. Accordingly, hemoglobin is present only in red blood cells. It is now well established that differential gene expression results in the carefully controlled (or regulated) expression of functional proteins, such as hemoglobin and insulin.

Gene expression is used for studying gene function. Genes are now routinely expressed in cultured cell lines by using viral vectors carrying cDNA, the transcription of which yields the gene's mRNA. RNA-RNA interaction can induce gene expression and RNA can regulate its activities without necessarily requiring a protein. The protein produced from mRNA may confer specific and detectable function on the cells used to express the gene. It is also possible to manipulate cDNA so that proteins are expressed in a soluble form fused to polypeptide tags. This allows purification of large amounts of proteins that can be used to raise antibodies or to probe protein function in vivo in animals. Knowledge of which genes are expressed in healthy and diseased tissues would allow us to identify both the protein required for normal function and the abnormalities causing disease. This information will help in the development of new diagnostic tests for various illnesses as well as new drugs to alter the activity of the affected genes or proteins.

### DNA Sequences and Structure

The human genome project has provided the genetic sequence of the entire human genome and identified the need for further work to study the biological function of genes. X-ray crystallography has been used to determine the 3D structures of nearly all the possible sequences of DNA at atomic level and create a map of DNA structure. This will help to explain function of genes and gene expression, which often occurs through variations in DNA structure and may provide answers to questions as to why some DNA structures are inherently prone to damage or mutation and how DNA is able to repair itself. An understanding of DNA structure and its relationship to genetic sequences will advance applications in molecular diagnostics, gene therapy, nanobiotechnology and other areas of biomedicine.

**Table 1.3** Genetic variations in the human genome

| Variation | Features |
|---|---|
| Complex chromosomal rearrangements (CCRs) | CCRs account for a large fraction of non-recurrent rearrangements at a given locus |
| Copy number variation (CNV) | DNA segments >1 kb in length, whose copy number varies with respect to a reference genome. ~12 % of human genes vary in DNA sequences they contain |
| Insertions and deletions in the human genome (INDEL) | INDELS are an alternative form of natural genetic variation that differs from SNPs |
| Interspersed repeated elements | Long and short interspersed nuclear elements are a significant portion of human genome |
| Large scale variation in human genome | Large portions of DNA can be repeated or missing for no known reason in healthy persons |
| Segmental duplication | Duplicons have >90 % sequence homology to another region in the genome |
| Single nucleotide polymorphisms (SNP) | SNPs are sequence variations at single base pair level with a population frequency of >1 % |
| Structural variations (SVs) | SVs involve kilobase- to megabase-sized deletions, duplications, insertions, inversions, and complex combinations of rearrangements |
| Tandem repeats | Tandem sequences repetitions represent ~10 % of the genome |



## *Genetic Variations in the Human Genome*

Human genome rearrangements can occur by several mechanisms that include both recombination and replication-based mechanisms. The latter can result in complex genomic rearrangements. Genetic variations in the human genome are listed in Table 1.3.

### Single Nucleotide Polymorphisms

Small stretches of DNA that differ in only one base are called single nucleotide polymorphisms (SNP) and serve to distinguish one individual's genetic material from that of another. SNPs comprise some 80 % of all known polymorphisms. Millions of SNPs have been discovered in humans, and are available in public databases. Each gene contains approximately five coding SNPs, which likely effect the expression of the currently estimated 20,000 genes. Identification of SNPs is important as it helps in understanding the genetic basis of common human diseases. In the absence of functional information about which polymorphisms are biologically significant, it is desirable to test the potential effect of all polymorphisms on drug response. Technologies for SNP genotyping are described in Chap. 5. Potential uses of SNP markers are:

- Genome analysis for linkage studies
- Genome scan for association studies
- Candidate gene mapping

- Drug discovery
- Prediction of adverse effects of drugs
- Prediction of drug efficacy

### Copy Number Variations in the Human Genome

Copy number variation (CNV) refers to variation from one person to another in the number of copies of a particular gene or DNA sequence (usually exceeding 1,000 bp). CNV is a source of genetic diversity in humans. Numerous CNVs are being identified with various genome analysis platforms, including array comparative genomic hybridization (aCGH), SNP genotyping platforms, and next-generation sequencing (NGS). CNV formation occurs by both recombination-based and replication-based mechanisms and de novo locus-specific mutation rates appear much higher for CNVs than for SNPs. CNVs can cause Mendelian or sporadic traits, or be associated with complex diseases by various molecular mechanisms, including gene dosage, gene disruption, gene fusion, position effects, etc. However, CNV can also represent benign polymorphic variants. CNVs, especially gene duplication and exon shuffling, can be a predominant mechanism driving gene and genome evolution. CNVs form at a faster rate than other types of mutation, and seem to do so by similar mechanisms in bacteria, yeast and humans. A review of some models of the mechanisms that cause CNV reveals that although non-homologous end-joining mechanisms are well known, the focus is on perturbation of DNA replication and replication of non-contiguous DNA segments, e.g. cellular stress might induce repair of broken replication forks to switch from high-fidelity homologous recombination to non-homologous repair, thus promoting CNV (Hastings et al. 2009).

CNV of DNA sequences is functionally significant but has yet to be fully ascertained. An international team of investigators has published a study showing that ~12 % of human genes vary in the CNV of DNA sequences they contain – a finding that contradicts the statement that the DNA of any two humans is 99.9 % similar (Redon et al. 2006). The discovery indicates that CNV could play a larger role in genetic disease than previously thought, with broad implications in disease association studies, genetic diagnostic testing, and cancer research. The investigators constructed a first-generation CNV map of the human genome through the study of 270 individuals from four populations with ancestry in Europe, Africa or Asia (the HapMap collection). DNA from these individuals was screened for CNV using two complementary technologies: SNP genotyping arrays, and clone-based comparative genomic hybridization (CGH). A total of 1,447 copy number variable regions (CNVRs), which can encompass overlapping or adjacent gains or losses, covering 360 megabases (12 % of the genome) and 6–19 % of any given chromosome were identified in these populations. These CNVRs contained hundreds of genes, disease loci, functional elements and segmental duplications. Notably, the CNVRs encompassed more nucleotide content per genome than SNPs, underscoring the importance of CNV in genetic diversity and evolution. The data obtained delineate linkage

disequilibrium patterns for many CNVs, and reveal marked variation in copy number among populations. They also demonstrated the utility of this resource for genetic disease studies. Of the 2,900 copy number variations, 285 are already known to be associated with disease, including AIDS, inflammatory bowel disease, lupus, cataracts, arterial disease, and schizophrenia. The findings could change the direction of future genetic disease research, which has primarily focused on SNPs. Some diseases are caused by copy number variation rather than SNPs.

CNVs are still relatively under-ascertained. Tiling oligonucleotide microarrays, comprising 42 million probes, were used to generate a comprehensive map of 11,700 CNVs greater than 443 base pairs, of which most have been validated independently (Conrad et al. 2010). Reference genotypes were generated from 450 individuals of European, African or East Asian ancestry. The predominant mutational mechanisms differ among CNV size classes. Retrotransposition has duplicated and inserted some coding and non-coding DNA segments randomly around the genome. Furthermore, by correlation with known trait-associated SNPs, 30 loci with CNVs were identified that are candidates for influencing disease susceptibility. The results show that any two genomes differ by more than 1,000 CNVs, or approximately 0.8 % of a person's genome sequence. Most of these CNVs are deletions, with a minority being duplications. Two consequences are particularly striking in this study of apparently healthy people. First, 75 regions have jumped around in the genomes of these samples: second, more than 250 genes can lose one of the two copies in our genome without obvious consequences and a further 56 genes can fuse together potentially to form new composite genes. This map complements the cataloguing of SNPs delineated in the HapMap Project. In spite of the power of this map, the heritability void left by genome-wide association studies will not be accounted for by common CNVs in case of complex diseases such as diabetes or heart disease.

By analyzing short-read mapping depth for 159 human genomes, a study has demonstrated accurate estimation of absolute copy number for duplications as small as 1.9 kilobase pairs, ranging from 0 to 48 copies (Sudmant et al. 2010). Approximately 4.1 million "singly unique nucleotide" positions were identified, which provided information for distinguishing specific copies, and were used to genotype the copy and content of specific paralogs within highly duplicated gene families. These data identify human-specific expansions in genes associated with brain development, reveal extensive population genetic diversity, and detect signatures consistent with gene conversion in the human species. This approach enables access to ~1,000 genes for genetic studies of disease association.

### CNV Association with Disease

CNVs not only contribute to the phenotypic diversity among humans, they have been also been associated with disease susceptibility. Diseases associated with CNVs include AIDS, inflammatory bowel disease, lupus, cataracts, cardiovascular diseases, neurological diseases, autism and schizophrenia. The findings could change the direction of future genetic disease research, which has primarily focused on SNPs.

Although CNVs confer a risk of disease, they may not be sufficient by themselves to lead to a specific disease outcome, leading to speculation that additional risk factors may account for the variation. The factors underlying the phenotypic variation associated with seemingly identical genomic alterations have not been entirely clear and present challenges for clinical diagnosis, counseling, and management. A "two-hit," or second-site model is based on the observation that affected persons with a microdeletion on chromosome 16p12.1 are more likely to have additional large CNVs than are controls (Girirajan et al. 2010). These data supported an oligogenic basis, in which the compound effect of a relatively small number of rare variants of large effect contributes to the heterogeneity of genomic disorders, and provided testable predictions of the cause of syndromic disorders and those with phenotypic variation. In a further study, these authors observed considerable variation in the phenotypes associated with several recurrent specific CNVs that are relatively prevalent (Girirajan et al. 2012). This finding was complicated by the identification of apparently normal or mildly affected carrier parents with 16p11.2,15,17,18 1q21.1,32 or 16p12.121 CNVs, suggesting that these variants are critical but not sole determinants of phenotype. These data are consistent with locus heterogeneity and a modest number of high-impact variants contributing to a spectrum of disease severity within families. The interpretation of variants associated with phenotypic variation remains challenging at the clinical level, but this study provides help in understanding factors that contribute to the phenotypic outcome, which may be used for counseling. It explains why persons with the same chromosomal abnormality may have very different clinical outcomes: some of them may simply have a second genetic event that makes matters worse for them. The findings of the study represent a step toward deconvoluting the effect of CNVs in disease and understanding, more broadly, the causes of neurologic disease. The analysis shows that the phenotypic variation of some genomic disorders may be partially explained by the presence of additional large variants.

### Insertions and Deletions in the Human Genome

Emory University scientists have identified and created a map of 415,436 insertions and deletions (INDELs) in the human genome that signal a little-explored type of genetic difference among individuals (Mills et al. 2006). INDELS are an alternative form of natural genetic variation that differs from the much-studied SNPs. Both types of variation are likely to have a major impact on humans, including their health and susceptibility to disease.

SNPs are differences in single chemical bases in the genome sequence, whereas INDELs result from the insertion and deletion of small pieces of DNA of varying sizes and types. If the human genome is viewed as a genetic instruction book, then SNPs are analogous to single letter changes in the book, whereas INDELs are equivalent to inserting and deleting words or paragraphs. INDELs were discovered using a computational approach to re-examine DNA sequences that were originally generated for SNP discovery projects. INDELs are distributed throughout the

human genome with an average density of one INDEL per 7.2 kb of DNA. Variation hotspots were identified with up to 48-fold regional increases in INDEL and/or SNP variation compared with the chromosomal averages for the same chromosomes. The scientists expect to expand the map to between 1 and 2 million by continuing their efforts with additional human sequences. INDELs can be grouped into five major categories, depending on their effect on the genome:

1. Insertions or deletions of single base pairs
2. Expansions by only one base pair (monomeric base pair expansions
3. Multi-base pair expansions of 2–15 repeats
4. Transposon insertions (insertions of mobile elements)
5. Random DNA sequence insertions or deletions.

INDELs already are known to cause human diseases. For example, cystic fibrosis is frequently caused by a three-base-pair deletion in the CFTR gene, and DNA insertions called triplet repeat expansions are implicated in fragile X syndrome and Huntington's disease. Transposon insertions have been identified in hemophilia, muscular dystrophy and cancer. INDEL maps will be used together with SNP maps to create one big unified map of variation that can identify specific patterns of genetic variation to help predict the future health of an individual. The next phase of this work is to figure out which changes correspond to changes in human health and develop personalized health treatments. All the INDELs identified in the study have been deposited into dbSNP – a publicly available SNP database hosted by the National Center for Biotechnology Information. The National Human Genome Research Institute of the NIH funded the research.

GeneVa™ structural genomic variations platform (Compugen) provides predicted non-SNP, medium and large-scale genetic variations in the human genome. Currently, it incorporates a database – developed during the past year – of approximately 200,000 novel predicted insertions, deletions and copy-number variations in the human genome. This database was created by analyzing genomic, EST (Expressed Sequence Tag), disease related and other databases. A specialized computational biology analysis platform was developed to handle and integrate these disparate data sources, identify possible genomic structural variations and predict their association with specific disease pathways such as those associated with breast and colon cancer, diabetes type II and Parkinson's disease.

### Large Scale Variation in Human Genome

Large-scale disparities in the DNA of healthy people have been revealed, which challenge the previous findings, and reveal a largely ignored source of genome variation. This finding implies that healthy persons can have large portions of DNA that are repeated or large portions that are missing for no known reason. This previously unappreciated heterogeneity may underlie certain human phenotypic variation and susceptibility to disease and argues for a more dynamic human genome structure.

The size of genomes isolated from mouse liver tissues increases with age, peaking at 5 weeks and the copy number of several retro-element sub-families are up to twofold higher in liver tissue than in lung or spleen tissue (Lee et al. 2012). The findings that the genome structure of an individual is variable depending on age and organ type in association with the transposition of retroelements may have broad implications in understanding biologic phenomena. Data from this study indicate that there may be multiple variant isoforms of an individual. This finding indicates that a new protocol or system and more research will be needed to analyze and make sense of how the structural changes in the genome relate to an individual's health. It has implications for personalized medicine. Further work is required to pinpoint which structural changes in the genome correlate to a particular disease process and this might eventually provide clinicians with new prognostic biomarkers.

## Structural Variations in the Human Genome

Structural changes are extremely common in human populations. Genetic variation among individual humans occurs on many different scales, ranging from gross alterations in the human karyotype to a SNP. More bases are involved in structural changes in the genome than are involved in single-base-pair changes.

Although the original human genome sequencing effort was comprehensive, it left regions that were poorly analyzed. Later investigations revealed that, even in healthy individuals, many regions in the genome show structural variations (SVs), which involve kilobase- to megabase-sized deletions, duplications, insertions, inversions, and complex combinations of rearrangements. A study offers a new view of what causes the greatest genetic variability among individuals – suggesting that it is due less to single point mutations than to the presence of structural changes that cause extended segments of the human genome to be missing, rearranged or present in extra copies (Korbel et al. 2007). This study was designed to fill in the gaps in the genome sequence and to create a technology to rapidly identify SVs between genomes at very high resolution over extended regions. A novel DNA-based method called Paired-End Mapping was used for this study. Researchers broke up the genome DNA into manageable-sized pieces about 3,000 bases long; tagged and rescued the paired ends of the fragments; and then analyzed their sequence with a high-throughput, rapid-sequencing method developed by 454 Life Sciences. This method of sequencing can generate hundreds of thousands of long read pairs, which are unique within the human genome, to quickly and accurately determine genomic variations. Whereas previous studies, based on point mutations estimated that there is a 0.1 % difference between individuals, this work points to a level of variation between 2 and 5 times higher. There were 'hot spots', i.e. regions with a lot of variation, which are often regions associated with genetic disorder and disease. These results will have an impact on how genetic effects in disease are studied. It was previously assumed that 'landmarks,' like the SNPs, were fairly evenly spread out in the genomes of different people. Now, one has to take into account the SVs can distort the map and differ between individual patients. Even in healthy persons,

there are variants in which part of a gene is deleted or sequences from two genes are fused together without destroying the cellular activity with which they are associated. These findings show that the parts list of the human genome may be more variable and flexible than previously considered. The discovery of CNVs in our genomes has dramatically changed our perspective on DNA structural variation and disease.

### Mapping and Sequencing of Structural Variation from Human Genomes

The first high-resolution map showing the structural variants (SVs) that exists in the human genome has been published (Kidd et al. 2008). Using a clone-based method, the complete DNA sequences of eight people of diverse geographic ancestry was examined: four of African descent, two of Asian descent, and two of western European descent. The DNA sequence of those eight persons was compared to the DNA sequence derived from the Human Genome Project, which is known as the reference sequence. This map provides a comprehensive picture of the normal pattern of SV present in these genomes, refining the location of 1,695 SVs that more than about 6,000 base pairs long; 50 % of these were seen in more than one individual and lay outside regions of the genome previously described as structurally variant. The researchers discovered 525 new insertion sequences, ranging in size from a few thousand to 130,000 base pairs, which are not present in the human reference genome, and many of these are variable in copy number between individuals. Complete sequencing of 261 SVs revealed considerable locus complexity and provides insights into the different mutational processes that have shaped the human genome.

In various parts of human genome, some people have segments of DNA sequence that other people do not have. Large genetic regions may be flipped in one person compared with another and these differences can influence a person's susceptibility to various diseases. These data provide a standard for genotyping platforms and a prelude to future individual genome sequencing projects. The results also indicate that the human genome sequence is still incomplete that sequencing of additional genomes will be required to fill the remaining gaps. The eight people studied are part of a much larger group whose genomes will be sequenced as part of the 1,000 Genomes Project, an international effort to sequences the genomes of people from around the world.

In order to understand structural variation, it is also essential to develop new technologies designed to detect genetic differences among people. For example, SNP biochips, whether used in research or in clinical applications, need to reflect this structural variation to find links between particular gene variants and diseases. Currently available biochips would miss an association for nearly half of these sites. Besides their potential applications, the new results provide a wealth of data to explore hypotheses and make discoveries as we now have eight new reference human genomes.

The SV study used custom Agilent microarrays to assess the copy number status of the unannotated sequences by array comparative genomic hybridization (aCGH). More than 40 % of the novel sequences showed CNV. This map of human SV is highly consistent with previous high-resolution CNV studies that found a considerably smaller size distribution for CNV regions compared to studies that employed bacterial artificial chromosome (BAC)-based aCGH, and predicts that the current database of CNV is overstated. The study's clone-based method enabled mapping and complete sequencing of many CNV regions, enabling valuable insights into the mechanisms that mediate human SV.

### Role of DNA Sequencing in the Development of Personalized Medicine

Molecular diagnostics and DNA sequencing are among the important basics of personalized medicine. Role of sequencing in synthetic biology for drug discovery and development is discussed later in this chapter. Personalized genome sequencing would become an integral part of personalized medicine as the cost comes down. Sequencing will also lead to the development of many diagnostic assays that will contribute to personalized medicine. Simple-to-operate and affordable small sequencers can be integrated in point-of-care diagnostics for personalized medicine.

Availability of low-cost genomic sequencing will expand the use of genomic information in the practice of medicine. Drugs will be targeted better to diseases in particular patients based on genotype information. Toxicity will be predictable in most cases prior to drug administration. By the end of the second decade of the twenty-first century, it is anticipated that the general population will have the opportunity to carry a chip card, like a credit card, with all the genetic information of the person coded on it. Such a database can be constructed by taking a blood sample of the individual, resequencing the functional DNA, and identifying the genetic variations in functional genes.

## *Interconnected Genetic and Genomic Patterns in Human Diseases*

According to a unified genomic model of human disease, human diseases are caused by alleles that encompass the full range of variant types, from chromosomal rearrangements and CNVs down to the individual SNPs, and these variations span a broad frequency spectrum, from the very rare to the common. The picture emerging from analysis of whole-genome sequences, the 1,000 Genomes Project pilot studies, and targeted genomic sequencing derived from very large sample sizes reveals an abundance of rare and private variants (Lupski et al. 2011). One implication of this realization is that recent mutation may have a greater influence on disease susceptibility or protection than is conferred by variations that arose in distant ancestors.

According to the authors, the most important thing is not to focus disproportionately on specific variants, but rather to integrate across all classes or risk-associated variants. In some individuals, risk may be caused by an unusual combination of common variants, whereas in others it will be due to a smaller number of large effect rare variants. Nevertheless, the extent to which private or rare genetic variation are turning up in large-scale genome sequencing studies, personal genome analyses, and targeted gene sequencing work hints that these mutations have a previously unappreciated influence over traits and diseases. There is considerable medically actionable information that can be gleaned from genetic and genomic studies of these recent mutations in the genome that are shared between family members. The authors state that this "clan genomics" model could help in interpreting personal genome and disease data. This can be leveraged to promote a better gene discovery. Another goal of the study was to encourage a move away from a preoccupation with accounting for all of the heritability for a given disease. It is not necessary to account for all of the heritability in order to better understand biology and improve human health. It is also important to consider the influence that rare and common variants can have on one another, because each personal genome has a collection of deleterious as well as protective variations, which in combination dictate the health of the individual. Considering common diseases involving many genes and Mendelian diseases associated with high penetrance, rare genetic variants are not necessarily separate entities, since they sometimes involve different types of alterations to the same genes or pathways. Common variations in the so-called Mendelian disease genes are also contribute to more common chronic disease in the population.

## Basics Technologies for Developing Personalized Medicine

### *Definitions of Technologies Relevant to Personalized Medicine*

Important basics of personalized medicine are derived from the following technologies and approaches, which will be described in more detail in various chapters of the report:

1. Molecular diagnostics, particularly single nucleotide polymorphism genotyping
2. Integration of diagnostics with therapy, particularly monitoring of therapy
3. Bioinformatics for evaluation and use of data from various biotechnologies
4. Pharmacogenomics is the application of genomics (variations of DNA as well as RNA) to drug discovery and development. It involves the study of mechanism of action of the drugs on the cells as revealed by gene expression patterns.
5. Pharmacogenetics is a term recognized in pharmacology in the pre-genomic era and concerns the study of influence of genetic factors on response to drugs. With advances in genomics, role of gene polymorphisms on action of drugs has been added to this.

6. Pharmacoproteomics is the application of proteomics to drug discovery and development. Discovery of protein biomarkers may serve as a common basis of diagnostics and therapeutics. Subtyping patients on the basis of protein analysis may help to match a particular target-based therapy to a particular marker in a subgroup of patients.
7. Pharmacometabolomics is the application of metabolomics for study of diseases, discovery of biomarkers, for development of diagnostics and therapeutics.

### *Problems with the ICH Definitions of Pharmacogenomics and Pharmacogenetics*

The International Conference on Harmonization (ICH) finalized a set of definitions that were published as a guideline in 2008 for use by international scientists, companies, and regulators in assessing pharmacogenomics products and services. These have not been changed since then.

- ICH defined pharmacogenomics as "the study of variations of DNA and RNA characteristics as related to drug response."
- Pharmacogenetics was described as a sub-set of pharmacogenomics, for "the study of variations in DNA sequence as related to drug response."

The ICH started the project to remedy the inconsistency of applied definitions, which could lead to conflicting usage and interpretations by regulators, industry, investors, and ethics groups. However, the definition of pharmacogenetics will complicate the situation as it is erroneous. The main reasons for this are:

- Pharmacogenetics existed long before pharmacogenomics and cannot be a subset of genomics any more than genetics can be a subset of genomics.
- Pharmacogenetics takes into consideration many factors other than variations in DNA sequences in determining the response to drugs (see Chap. 3).

## 'Omics' and Personalized Medicine

Starting as the suffix of genomics, 'omics' refers to nearly 100 technologies, all of which are relevant to development of personalized medicine. Some of the important 'omics' are described in various chapters of this report. The Genome Institute of Singapore has launched the POLARIS Program (Personalized OMIC Lattice for Advanced Research and Improving Stratification), a strategic initiative for embedding 'omics' information into the diagnosis and treatment of diseases in Singapore. The POLARIS consortium will establish certified labs at Singapore General Hospital as well as develop 'omics' platforms, technologies for processing clinical samples, bioinformatics for data interpretation/results analysis, and reporting to clinicians. It will facilitate the development of personalized medicine.

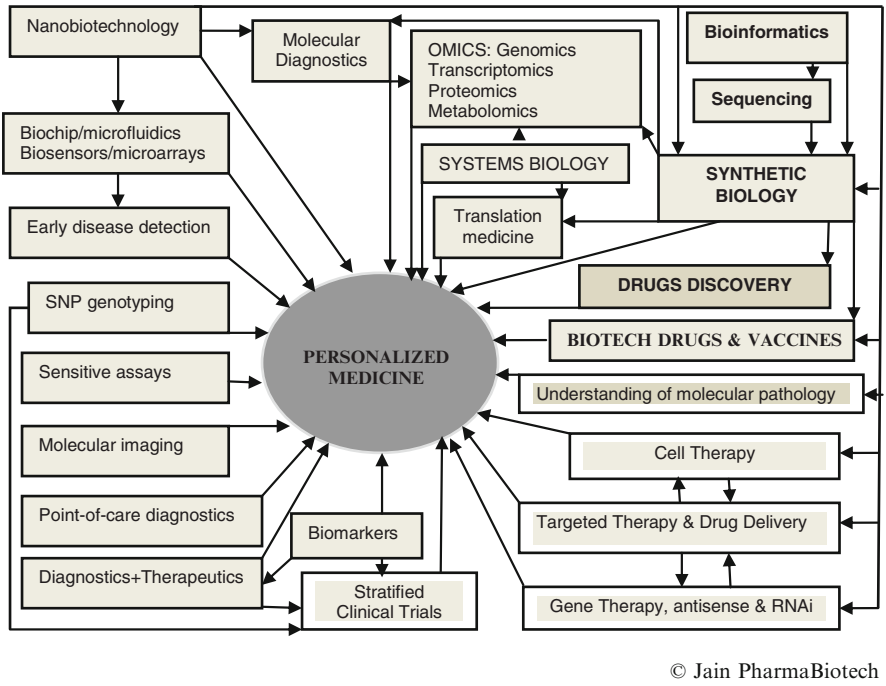


**Fig. 1.1** Relation of personalized medicine to other technologies

### *Relationship of Various Technologies to Personalized Medicine*

Relationship of various technologies to personalized medicine is shown in Fig. 1.1.

## Conventional Medicine Versus Personalized Medicine

Conventional medicines had a start as empirical therapies. Even as mechanism-based therapies started to develop, lack of efficacy and adverse effects were noted and accepted to a certain extent. Most of conventional medicines were developed as universal drugs for a certain disease. For diseases with multiple pharmacotherapies, the choice was usually left to the prescribing physician's experience and preferences. With the advances in pharmacogenetics, it became obvious that something could be done for the following problems with conventional medicines.

- Genetic variations among individuals lead to differences in response to drugs
- High percentage of lack of efficacy with some medicines
- High incidence of adverse effects to drugs

- Evidence-based medicine supports a standardized application of therapy that does not take into account variations of response in individual patients.
- Clinical trials are geared around taking statistical information about the general population of patients and applying it to the individual.

## Personalized Medicine and Evidence-Based Medicine

Guidelines for evidence-based medicine are generated from the highest level of evidence from multiple randomized controlled clinical trials to address a particular clinical problem. It has merits although it differs from personalized medicine. Randomized clinical trials have specific inclusion and exclusion criteria designed to represent a population broad enough and sufficiently enriched to attain a requisite number of end points and demonstrate a statistically and clinically significant difference in outcome. Development of evidence-based guidelines based on relatively broad enrolment criteria inhibits the subsequent development of personalized medicine within the enrolment criteria (Goldberger and Buxton 2013). Although claimed are made that evidence-based medicine has the care of individual patients as its top priority that these two approaches can be compatible, it is difficult to reconcile these concepts.

## Role of Genetics in Future Approaches to Healthcare

### *Genetic Medicine*

Genetics plays an important role in almost every disease. Our risk of contracting common diseases is generally thought to be determined largely by environment and lifestyle but there is strong epidemiological evidence that genes contribute to overall risk. In multiple sclerosis, for example, the siblings of an affected person have a 25-fold increase in risk of developing the disease compared with the general population. One may consider trauma to be unrelated to genetic factors but there are genetic factors leading to risk-prone behavior in some individuals and genetic factors may explain the variations in the body's response to an equivalent amount of trauma in various individuals.

Genetics is the study of single genes and their effects whereas genomics is the study not only of single genes, but also of the functions and interactions of all the genes in the genome. Sequencing of the human genome has increased the activity in genetic medicine. Genetic medicine is already beginning to enter the realms of primary care through the availability of testing for predisposition to certain cancers and carrier screening and diagnostic tests for common recessive disorders such as cystic fibrosis and hereditary hemochromatosis. This involvement will broaden as personalized medicine develops and pharmacogenetics will become increasingly

relevant in decisions about prescribing. Ultimately, pharmacogenetics may be a much greater driving force for the application of genetic medicine in primary care than specific genetic screening programs. Genetics will not remain the exclusive prerogative of specialist centers but every physician will need to use genetic knowledge to aid prescribing and clinical management.

## *Human Disease and Genes*

The Human Gene Nomenclature Committee defines a gene as "a DNA segment that contributes to phenotype/function". In the absence of demonstrated function, a gene may be characterized by sequence, transcription or homology. For practical purposes, a gene is a physical and functional unit of heredity, which carries information from one generation to the next. In molecular terms, it is the entire DNA sequence including exons, introns, and noncoding transcription control regions that are necessary for production of a functional protein or RNA.

The sequencing of the human genome has revealed considerable information to study the genetic basis of disease. The identification of all human genes and their regulatory regions provides the framework to expedite our understanding of the molecular basis of disease. More than 1,000 human genes have been implicated in specific diseases in the database of Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/Omim/). It is expected that the causative lesions in most monogenic diseases (resulting from mutation in a single gene) will be characterized in the next few years. Geneticists are now using sophisticated methods to track genes in polygenic disorders (caused by defects in more than one gene). Even though genes and proteins related to a disease are discovered, the underlying mechanism of how these genes cause the disease is not always understood. The study of model organisms often provides the first clues to the identity of a genetic defect in human disease. Sequencing of the genomes of some model organisms has provided an opportunity to use comparative genomics to study gene function. Along with *Caenorhabditis elegans*, zebrafish and other small creatures, the fruit fly has now entered a new stage of discovery, in which modeling of specific cellular pathways implicated inhuman disease may contribute to the search for new treatments.

## *Genetic and Environmental Interactions in Etiology of Human Diseases*

Most common diseases are caused by the interplay of genes and environment, with adverse environmental exposures acting on a genetically susceptible individual to produce disease. In contrast to single gene disorders such as cystic fibrosis, genes underlying common diseases are likely to be multiple, each with a small effect, but act in concert or with environmental influences to lead to clinical disease.

Genome-wide association studies (GWAS) have identifying approximately several loci for common diseases and traits. These associations provided new insights into pathophysiology, suggesting previously unsuspected causal pathways for common diseases that will be of use in identifying new therapeutic targets and developing targeted interventions based on genetically defined risks. Although GWAS have identified numerous loci contributing to common diseases, these explain a small fraction of the genetic component in disease. Likely explanations are relatively rare variations that are missed by such studies and the presence of gene-gene interactions.

Gene by environment (GxE) interactions is also important in many human diseases. Given the difficulty of assessing environmental factors in typical human studies, the presence of GxE interactions would reduce ability to detect important susceptibility loci. GxE interactions affecting gene expression may be a common and important contribution to complex diseases. Genetic analysis of thousands of transcript abundance traits in human primary endothelial cell lines in response to proinflammatory oxidized phospholipids implicated in cardiovascular disease revealed that approximately one-third of most regulated transcripts, showed evidence of GxE interactions (Romanoski et al. 2010). The interactions resulted primarily from effects of distal-, trans-acting loci, but a striking example of a local-GxE interaction was also observed for FGD6. Some of the distal interactions were validated by siRNA knockdown experiments. These findings add to the understanding of the overall architecture of complex human traits and are consistent with the possibility that GxE interactions are responsible, in part, for the failure of association studies to more fully explain common disease variation.

## *Role of Genetics in Development of Personalized Medicines*

Advances in genetics will also help in understanding drug action pathways, identifications of new targets, target validation and in silico screening. Companies that incorporate both genetics and genomics in the drug discovery process will be the ones to discover the innovative drugs of the future.

### Genetic Databases

Several genetic databases, governmental as well as private, are being developed and bring together streams of data about individuals. The best known of these is the Icelandic health sector database, managed by deCODE Genetic Inc in Iceland. Such databases include molecular genetic data, clinical data, lifestyle data, and genealogical data. Searching for causal associations between genetic and health phenomena is not new. Considerable data has been collected on the classic mendelian disorders and is used for patient care and counseling. The Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/Omim) has a catalogue of genes and

phenotypes. GeneClinics (http://www.geneclinics.org/) helps clinicians to relate the information from genetic testing to the diagnosis, management, and genetic counseling of patients and families with specific inherited diseases.

Advances in biotechnology enable us to obtain information on genetic makeup with speed, precision and at reasonable cost. Genetic details can be correlated with other complex information via computers. Genetic databases are now helping elucidate gene function, estimate the prevalence of genes in populations, differentiate among subtypes of diseases, trace how genes may predispose to or protect against illnesses, and improve medical intervention. They will play an important role in development of personalized medicine.

Genetic databases can be probed for gene-related variabilities in drug responsiveness and metabolism to tailor drugs to particular constitutions and to screen for genetic suitability before prescribing. Diseases in which genetic information has been studied for this purpose include asthma, migraine, Alzheimer's disease, depression, psoriasis, and osteoarthritis. Pharmaceutical and biotechnology companies are either building or buying access to genetic databases and DNA libraries, often based on data from clinical trials.

### Clinical Genomic Database

Although technological advances have greatly increased the availability of human genomic sequencing, the capacity to analyze genomic data in a clinically meaningful way lags behind the ability to generate such data. To address this obstacle, all conditions with genetic causes were reviewed to constructed the Clinical Genomic Database (CGD), a searchable, freely Web-accessible (http://research.nhgri.nih.gov/CGD/) database of conditions based on the clinical utility of genetic diagnosis and the availability of specific medical interventions (Solomon et al. 2013). CGD currently includes 2,616 genes organized clinically by affected organ systems and interventions (including preventive measures, disease surveillance, and medical or surgical interventions) that could be reasonably warranted by the identification of pathogenic mutations. To aid independent analysis and optimize new data incorporation, CGD also includes all genetic conditions for which genetic knowledge may affect the selection of supportive care, informed medical decision-making, prognostic considerations, reproductive decisions, and allow avoidance of unnecessary testing, but for which specific interventions are not otherwise currently available. For each entry, the CGD includes the gene symbol, conditions, allelic conditions, clinical categorization (for both manifestations and interventions), mode of inheritance, affected age group, description of interventions/rationale, links to other complementary databases, including databases of variants and presumed pathogenic mutations, and links to PubMed references (>20,000). The CGD will be regularly maintained and updated to keep pace with scientific discovery. Further content-based expert opinions are actively solicited. Eventually, the CGD may assist the rapid curation of individual genomes as part of active medical care.

## Genetic Epidemiology

Genetic epidemiology is the study of the etiology, distribution, and control of disease in groups of relatives and of inherited causes of disease in populations. From its parent disciplines of genetics and epidemiology, it has inherited the key elements of studying defined populations while investigating the roles of genes and the environment in relation to each other and endeavoring to account for the known biology of diseases. Quantifying the risk associated with genetic variation is a prerequisite for assessing the use of this new knowledge in medicine.

Research in disease etiology has shifted towards investigating genetic causes, powered by the human genome project. Successful identification of genes for monogenic disease has led to interest in investigating the genetic component of diseases that are often termed complex that is, they are known to aggregate in families but do not segregate in a mendelian fashion. Genetic epidemiology has permitted identification of genes affecting people's susceptibility to disease. While the role of genetic factors in diseases such as hypertension, asthma, and depression are being intensively studied, family studies and the large geographical and temporal variation in the occurrence of many diseases indicate a major role of the environment. Thus, it is necessary to consider findings about susceptibility genes in the context of a population and evaluate the role of genetic factors in relation to other etiological factors. Several approaches have been used to resolve the genetics of disease and to study the relation of genes to environmental factors in the population. Until now, population screening involving genetics has focused on the identification of persons with certain mendelian disorders before the appearance of symptoms and thus on the prevention of illness. In the future, we are likely to screen entire populations or specific subgroups for genetic information in order to target intervention in individual patients for the purpose of prevention of disease.

## Limitations of Medical Genetics and Future Prospects

Some of the limitations of investigations into the genetic basis of disease are:

1. Shortage of medical geneticists.
2. Disease phenotypes have been under-appreciated by geneticists. Ideally investigators should initially study phenotypes without knowing genotypes to ensure that the latter does not unduly influence the analysis of the former.
3. Extended pedigrees of affected families have not been studied adequately.
4. Genetic linkage studies often have different, even conflicting results. There is need for multiple groups to collaborate and pool their data to discover the part of the genetic "signal" on which they can agree.
5. Statistical methods for study of medical genetics need to be greatly improved.
6. Genetic variants involved in common diseases are of low to moderate penetrance, i.e., only some carriers will develop the disease. Many of these moderately-penetrant gene variants may be difficult to detect using classical methods of genetic research. New methods need to be specifically designed to identify these types of gene variants. This information can be used to improve healthcare through disease risk-reduction, earlier diagnosis and more specific therapies.

## Role of Systems Biology in Personalized Medicine

Systems biology is defined as the biology of dynamic interacting networks. It is also referred to as pathway, network, or integrative biology. An analysis of the structure and dynamics of network of interacting elements provides insights that are not obvious from analysis of the isolated components of the system. This requires that the biological frontiers drive the development of new measurement and visualization technologies and the pioneering of new computational and mathematical tools – all of which requires a cross-disciplinary environment composed of biologists, chemists, computer scientists, engineers, mathematicians, physicists, and physicians speaking common discipline languages. The combination of high-throughput methods of molecular biology with advanced mathematical and computational techniques has made it possible to screen and analyze the expression of entire genomes, simultaneously assess large numbers of proteins and their prevalence, and characterize in detail the metabolic state of a cell population. Complementing large-scale assessments, there are more subtle analyses that rationalize the design and functioning of biological modules in exquisite detail. This intricate side of systems biology aims at identifying the specific roles of processes and signals in smaller, fully regulated systems by computing what would happen if these signals were lacking or organized in a different fashion. The elucidation of this system requires high-precision, dynamic in vivo metabolite data, combined with methods of nonlinear systems analysis, and may serve as a paradigm for multidisciplinary approaches to fine-scaled systems biology.

The emergence of systems biology is bringing forth a new set of challenges for advancing science and technology. Defining ways of studying biological systems on a global level, integrating large and disparate data types, and dealing with the infrastructural changes necessary to carry out systems biology, are just a few of the extraordinary tasks of this growing discipline. Despite these challenges, the impact of systems biology will be far-reaching, and significant progress has already been made. Moving forward, the issue of how to use systems biology to improve the health of individuals must be a priority. It is becoming increasingly apparent that the field of systems biology will have a major role in creating a predictive, personalized, preventive, and participatory (P4) approach to medicine (Galas and Hood 2009). It will also facilitate the transfer of technologies relevant to personalized medicine from preclinical to clinical phase.

Systems biology can facilitate the development of personalized medicine by identification of the biological networks in which SNPs associated with the response to therapy exert their influence. It may help in determining how SNPs modify key biological processes such as cell differentiation, apoptosis and cell communication. Identification of the role of multiple SNPs in modifying the function of signaling pathways, which are implicated in complex disease pathogenesis, may enable development of interventions that are required to change from the non-responder to the responder status of a patient.

The National Institute of General Medical Sciences (NIGMS), which has created two National Centers for Systems Biology in the US, has defined systems biology as "an integrated experimental, informational, and computational science" that has "benefited from advances in genomics, proteomics, metabolomics, and other high-throughput technologies and is driven by innovations in computational analysis and simulation." These centers will study systems biology systems, multi-scale modeling approaches, signaling, genetic, and metabolic networks, and genetic variations in relation to complex phenotypes. Systems biology concept has been applied to other sciences relevant to personalized medicine: systems pathophysiology of diseases and systems pharmacology.

## *Systems Pharmacology*

Systems pharmacology seeks to develop a global understanding of the interactions between pathophysiology and drug action (Wist et al. 2009). It will enable an understanding of adverse effects of drugs by considering targets in the context of the biological networks in which they exist. Experimental and computational approaches allow systems pharmacology to obtain holistic, mechanistic information on disease networks and drug responses, and to identify new drug targets and specific drug combinations. Network analyses of interactions involved in pathophysiology and drug response across various scales of organization, from molecular to organismal, will enable the integration of the systems-level understanding of drug action and enable drug discovery for personalized medicine. Systems pharmacology will integrate pharmacogenetics, pharmacogenomics and pharmacoproteomics, which will be described in later chapters. Figure 1.2 shows relation of systems pharmacology to personalized medicine.

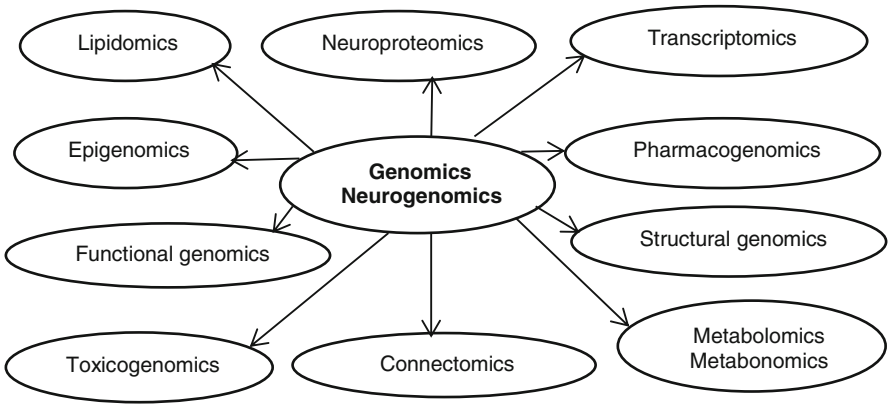


**Fig. 1.2** Relation of systems pharmacology to personalized medicine

## *Systems Medicine*

The concept of systems biology is applied to systems medicine and is relevant to personalized medicine. Computational and mathematical tools have enabled the development of systems approaches for deciphering the functional and regulatory networks underlying the behavior of complex biological systems. Further conceptual and methodological developments of these tools are needed for the integration of various data types across the multiple levels of organization and time frames that are characteristic of human disease. Because of the complexity of cellular networks that are perturbed in human disease development and progression as well as complex interactions between different cells of the human body, it is difficult to determine the molecular cause of disease in an individual patient. Even though considerable data has accumulated from "omics" studies in different tissues of large patient cohorts, systems medicine approach is needed for integration of these data and simulation of biological networks to study their dynamic behavior (Nielsen 2012).

Medical genomics has attempted to overcome the initial limitations of genome-wide association studies and has identified a limited number of susceptibility loci for many complex and common diseases. Systems approaches are starting to provide deeper insights into the mechanisms of human diseases, and to facilitate the development of better diagnostic and prognostic biomarkers for cancer and many other diseases. Systems approaches will transform the way drugs are developed through academic-industrial partnerships that will target multiple components of networks and pathways perturbed in diseases. In addition to better insight into mechanism of action of existing drugs, new drug targets will be identified, and development of drugs unlikely to be successful can be terminated at an early stage before expensive clinical development.

Advances in genomics and other -omics during the last decade have resulted in unprecedented volumes of complex data, which can enable physicians to provide their patients with more personalized care. Some of the expertise needed for understanding and management this data is lies outside the scope of conventional medical practice; therefore multidisciplinary collaboration coupled to a systems approach is important for exploiting its potential. Systems medicine builds on the successes in the field of systems biology by recognizing the human body as a multidimensional network. Systems medicine provides a conceptual and theoretical basis with the goal to provide physicians the tools necessary for translating the rapid advances in basic biomedical science into their routine clinical practice (Vandamme et al. 2013).

Major non-communicable diseases (NCDs) – cardiovascular diseases, cancer, chronic respiratory diseases, diabetes, rheumatologic diseases and mental health – represent the predominant health problem of the twenty-first century. Trend in the management of NCDs is a holistic integrative approach. A health system built around systems medicine and strategic partnerships has been proposed to combat NCDs (Bousquet et al. 2014).

Systems approach will enable medicine to become predictive, personalized, preventive and participatory, and, in the process, concepts and methods from Western and Oriental cultures can be combined. It is recommended that systems medicine should be developed through an international network of systems biology and medicine centers dedicated to inter-disciplinary training and education, to help reduce the gap in healthcare between developed and developing countries.

## Synthetic Biology and Development of Personalized Medicines

Scientists at the J. Craig Venter Institute have reported the design, synthesis, and assembly of the genome starting from digitized genome sequence information and its transplantation into a recipient cell to create new bacterial cells that are controlled only by the synthetic DNA (Gibson et al. 2010). The researchers built up the synthetic genome of *Mycoplasma mycoides*, a fast-growing bacterium with a 1 million-base genome; by stitching together shorter stretches of DNA, each about 1,000 bases. They then transferred the completed genome into the shell of another bacterium *Mycoplasma capricolum* whose own DNA had been removed. The transplanted genome "booted up" the host cell and took over its biological machinery.

A central challenge of synthetic biology is to enable the growth of living systems using parts that are not derived from nature, but designed and synthesized in the laboratory. As an initial step toward achieving this goal, synthetic proteins have been created that can function in *E. coli*. Using a so-called binary code method that relies on strategic placement of polar and non-polar residues, a team of scientists has made more than a million stably folded strings of amino acids from genetic sequences distinct from those known to occur naturally (Fisher et al. 2011). They then screened these synthetic proteins in dozens of *E. coli* strains missing essential genes, identifying artificial proteins that could substitute for the organism's own proteins. They are molecular machines that function quite well within a living organism even though they were designed from scratch and expressed from artificial genes.

Advances in sequencing can be used for combining synthetic biology with personalized medicine. Synthetic biology will contribute to personalized medicine by introduction of therapeutic systems based on a synthetic genome, using an expanded genetic code, and designed for specific personalized drug synthesis as well as delivery and activation by a pathological signal (Jain 2013). The ability to control expression in target areas within a genome is important for use of synthetic biology to design personalized medicines. In Transcription Activator-Like (TAL) Effector Technology, 2 hypervariable amino acid residues in each repeat recognize

1 bp in the target DNA, and recognition sequences of TAL effectors are predictable (Boch et al. 2009). The modular protein architecture enabled the construction of artificial effectors with new specificities. TAL code is similar to a navigation system for the genome, allowing pinpoint delivery of functional control elements to any specified sequence. TAL Effector Technology (licensed by Life Technology) enables design of proteins to specifically target and bind to a desired sequence of DNA.

Availability of complete genome sequences, high throughput technologies and synthetic biology has enabled reverse vaccinology. Availability of sequence data from different specimens of the same species of a pathogen provides an opportunity to select novel candidates for personalized vaccines.

## Integration of Technologies for Personalized Medicine

The concept of personalized medicine is the best way to integrate all the cutting edge technologies for optimal application in healthcare as shown in the Fig. 1.3.

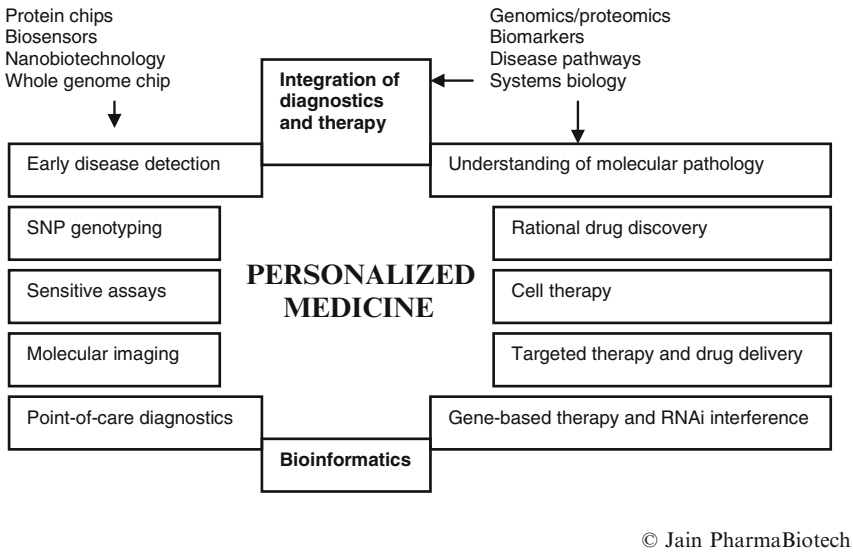


**Fig. 1.3** Integration of technologies for the development of personalized medicine

## Reclassification of Diseases

Because all major diseases have a genetic component, knowledge of genetic basis helps in distinguishing between clinically similar diseases. Classifying diseases based on genetic differences in affected individuals rather than by clinical symptoms alone makes diagnosis and treatment more effective. Identifying human genetic variations will eventually allow clinicians to subclassify diseases and individual therapies. Several diseases can now be described in molecular terms as some defects can give rise to several disorders. Reclassification of diseases on molecular basis rather than according to symptoms and gross pathology may enable the use of one drug to treat a number of diseases with the same molecular basis. Another way of reclassification of human diseases will be subdivision of patient populations within the same disease group according to genetic biomarkers and response to medications.

Many common diseases represent collections of different conditions each of which may have its own genetic cause. Advances in the diagnosis, treatment, and classification of human disease will depend on discovery of the function of each of the human genes. These genes will enable the sub-classification of diseases based on mechanism and clinical characteristics rather than symptoms alone. Taking into account the thousands of genes on each of the 23 chromosomes and the prediction that common diseases like diabetes and hypertension may be caused by three to one hundred different genes, this exciting process may well take several years of intense work by a global network of investigators working in universities and industry. This knowledge will revolutionize all aspects of medicine at the level of the patient and is relevant to the development of personalized medicine.

An example of the changing attitude towards the molecular basis of disease is the genetic basis of migraine, anxiety, and depression. This has been applied to discovery of the relevance of the dopamine receptor gene ($DR_{D2}$) to migraine. $DR_{D2}$ receptors are known targets of anti-emetic drugs used in migraine, and numerous polymorphisms have been identified in the $DR_{D2}$ gene. $DR_{D2}$ receptor antagonists have also been approved for the treatment of psychoses, anxiety, and depression. There is a genetic basis of the link between migraine, depression, and anxiety. The practical implications of this new information are the potential new indications for the numerous compounds that modulate the dopaminergic system and that are being developed only as neuroleptics. Clinical trials for the potentially new indications can be optimized by genotype analysis of patients with migraine, depression, and anxiety disorders.

Some variation in drug response may result from inadequate classifications of disease. For example, although two leukemias may appear identical morphologically, they may have different molecular profiles and thus respond differently to drug treatments. Without the molecular classification, the leukemias appear identical, and variation in response to the prescribed treatments would be highly unpredictable. More precise categorization of disease can potentially improve drug treatment by specifying which patients will respond to which treatments.

## Translational Science and Personalized Medicine

Translational science or medicine means applications of research findings for improving healthcare and is an important aspect of personalized medicine. It is defined as:

- T1 or translational phase 1 begins the translation journey from bench to bedside to community. During this phase, researchers usually conduct preclinical studies and phase I and II clinical trials.
- T2 expands discovery to larger patient populations in phase III and IV clinical trials, observational studies, and even some survey research.
- T3 launches the practice-oriented stage of translational research by implementing it to find out if a certain treatment or practice works in a real-world setting.
- T4 focuses on policy. If T1-T3 were successful, the next step is to find the best method of reaching clinicians and patients with a nationwide policy concerning treatment X or strategy Y.

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# Chapter 2
# Molecular Diagnostics in Personalized Medicine

## Introduction

Molecular diagnostics, the use of diagnostic testing to understand the molecular mechanisms of an individual patient's disease, will be pivotal in the delivery of safe and effective therapy for many diseases in the future. Diagnostics influence as much as 70 % of health care decision making, and a new generation of diagnostics tests that provide insights at the molecular level is delivering on the promise of personalized medicine. Role of molecular diagnostics in personalized medicine covers the following aspects:

- Early detection and selection of appropriate treatment determined to be safe and effective on the basis of molecular diagnostics
- Integration of molecular diagnostics with therapeutics
- Monitoring therapy as well as determining prognosis

In parallel with two important components of personalized medicine – pharmacogenetics and pharmacogenomics (compared in Table 5.2, Chap. 5) – there are two types of tests relevant to personalized medicine.

1. A pharmacogenomic test is an assay intended to study interindividual variations in whole genome SNP maps, haplotype markers or alterations in gene expression or inactivation that may be correlated with pharmacological function and therapeutic response. In some cases the pattern or profile of the change rather than the individual biomarker is relevant to diagnosis.
2. A pharmacogenetic test is an assay intended to study interindividual variations in DNA sequence related to drug absorption and disposition (pharmacokinetics), including polymorphic variations in genes that encode the functions of transporters, metabolizing enzymes, receptors and other proteins.

K.K. Jain, *Textbook of Personalized Medicine*, DOI 10.1007/978-1-4939-2553-7_2,

## Molecular Diagnostic Technologies

Molecular diagnostic technologies have been reviewed in a detailed report on this topic (Jain 2015c) Molecular diagnostics are used for genetic testing and have the potential to be applied for genetic screening of large populations. They can also be used as adjunct to clinical trials. Molecular diagnostic technologies relevant to personalized medicine are shown in Table 2.1. Some of these technologies used for

**Table 2.1** Molecular diagnostic technologies used for personalized medicine

| |
|---|
| **Polymerase chain reaction (PCR)-based methods** |
| Cold-PCR |
| Digital PCR |
| DirectLinear™ analysis |
| Quantitative fluorescent PCR |
| Real-time PCR |
| Reverse transcriptase (RT) PCR |
| Restriction fragment length polymorphism (RFLP) |
| Scorpions™: closed-tube platform for the efficient homogeneous detection of PCR amplicons |
| Single-strand conformational polymorphism |
| **Non-PCR methods** |
| Enzyme mutation detection |
| Fluorescense resonance energy transfer (FRET) based assays: Invader assay |
| Locked nucleic acid (LNA) technology |
| Peptide nucleic acid (PNA) technology |
| Transcription-mediated amplification |
| **Gene chip and microfluidic microarrays** |
| **Nanodiagnostics** |
| Nanoparticle-based integration of diagnostics with therapeutics |
| Nanotechnology-based refinement of diagnostics for pharmacogenetics |
| **Toxicogenomics** |
| **Single nucleotide polymorphism (SNP) genotyping** |
| **Copy number variations (CNV)** |
| **DNA methylation studies** |
| **Gene expression based tests** |
| **DNA sequencing** |
| Multiplex DNA sequencing |
| Next generation sequencing (NGS) |
| RNA sequencing |
| Whole exome sequencing (WES) |
| Whole genome sequencing (WGS) |
| **Cytogenetics** |
| Comparative genomic hybridization (CGH) |
| Fluorescent in situ hybridization (FISH) |

(continued)

**Table 2.1** (continued)

| |
|---|
| **Proteomic-based methods** |
| Fluorescent in situ protein detection |
| Protein/peptide arrays for identification of multiple biomarkers in blood and tissue samples |
| Protein biochip technology |
| Toxicoproteomics |
| **MicroRNA-based diagnostics** |
| **Single cell analysis** |
| Single cell PCR |
| Single cell proteomics |
| Single cell sequencing |
| Single cell gene expression |
| **Molecular imaging** |
| Functional magnetic resonance imaging (MRI) with nanoparticle contrast |
| Fluorodeoxyglucose positron emission tomography (FDG-PET) |
| Optical imaging |
| **Point-of-care (POC) diagnostics** |



mutation detection overlap with technologies for detection of single nucleotide polymorphisms (SNPs) described later in this chapter. The two most important technologies relevant to personalized medicine are single nucleotide polymorphism (SNP) genotyping and microarray/biochip.

## *PCR-Based Methods*

### DirectLinear™ Analysis

In DirectLinear™ Analysis, DNA molecules up to megabases in length are tagged with specific fluorophores and pass through a proprietary microfluidics system which stretches them to their full length, causing them to pass through and be read by the laser excitation and detection region in a linear fashion. The data generated is equivalent to a genetic "barcode" representing the spatial map of the fluorescent tags along the DNA. Each genomic barcode is unique to a specific individual or organism. DirectLinear™ Analysis has numerous potential applications in life science research and drug discovery as well as development. Entire genomes of novel organisms can be mapped nearly instantaneously, inviting comparison with known genomes and allowing researchers to focus on conserved regions or novel genomic features. Genetic differences between two samples or populations can readily be detected by comparing differences in barcode patterns, allowing the rapid identification of polymorphisms associated with disease or adverse drug response. Rapid genomic mapping of microbial organisms will have great utility in infectious disease research and diagnostics, as well as biodefense. Finally, rapid, low-cost access to each person's genomic information is a key to enabling molecular diagnostics and, ultimately, personalized medicine.

### Denaturing High-Performance Liquid Chromatography

Denaturing high-performance liquid chromatography (DHPLC) is a PCR-based method that uses liquid chromatography as an alternative to gel electrophoresis. DHPLC is traditionally used to detect variants in PCR products containing both allelic forms of a polymorphism (e.g. heterozygotes or a 1:1 mix of both alleles) via heteroduplex separation and thereby requires separate analyses of multiple individual test samples. DHPLC can be used to estimate absolute allele frequencies of SNPs in pooled DNA samples and also of the difference in allele frequency between different pooled DNA samples. This technique therefore offers an efficient and cheap method for genotyping SNPs in large case-control and family-based association samples.

### Multiplex Allele-Specific Diagnostic Assay

Multiplex Allele-Specific Diagnostic Assay (MASDA) employs a two-step approach to mutation detection. The first step is a DNA probe-based hybridization procedure in which DNA fragments from a patient sample are screened for the presence of specific mutations. In the second step, the hybridized probe is released from the patient's DNA sample, digested into fragments and identified by its unique banding pattern on gel electrophoresis. Identification of the probe reveals the mutation present in the sample DNA. MASDA has several applications in research as well as clinical laboratories. MASDA is also an important part of genetic profiling for pharmaceutical clinical trials.

### Representational Oligonucleotide Microarray Analysis

The power of representational oligonucleotide microarray analysis (ROMA) method is based on two fundamental realities: first, that the whole genome can be simplified into reproducible fragments sampled throughout the genome; second, that dense oligonucleotide arrays can quantify the amount of these fragments from different DNA sources. ROMA is ideally suited for the analysis of gene amplification and deletions in cancers. It is also an ideal technology to uncover genetic polymorphisms in normal populations represented by deletions and duplications.

### Restriction Fragment Length Polymorphism

Amplification of a specific region of the gene of interest by the PCR followed by digestion of amplified DNA products with restriction endonucleases is useful in screening for genetic mutations associated with altered metabolism of drugs or cancer susceptibility. Sizes of the digestion products from the study subjects are compared with those generated from a DNA substrate amplified from control subjects.

Difference in size is usually referred to as restriction fragment length polymorphism (RFLP). The size of the products is easily evaluated by agarose gel electrophoresis. PCR-based RFLP is used for identification of defined mutant alleles leading to complete functional deficiency of the enzyme CYP2D6.

#### Real-Time PCR for Detection of CNVs

TaqMan® Copy Number Assays (Life Technologies) are designed to detect and quantify CNVs and help researchers to better understand these processes by enabling them to determine CNVs from DNA samples, through a real-time PCR reaction. For example, study of the locations of CNVs in the human genome shows how they can be used as biomarkers for susceptibility to human diseases. The assays are used to validate the discovery of CNVs identified through the use of array-based platforms.

### *Non-PCR Methods*

#### Arrayed Primer Extension

Arrayed primer extension reaction (APEX) is a straightforward and robust enzymatic genotyping method in which hundreds to thousands of variations in the genome are simultaneously analyzed in a single multiplexed reaction. It differs from allele-specific hybridization in that the genotype information in APEX is obtained by single base extension, performed by a specific DNA polymerase, together with four different dye terminators (Pullat and Metspalu 2008). This approach ensures highly specific discrimination without allele-specific hybridization, because the primer to be extended anneals just adjacent to the DNA base that needs to be identified. Selection of primers for specific sites or their consecutive placement in tiled format, shifting them by one base, enables SNP analysis, mutation detection, or resequencing of the DNA template. It also permits the analysis of insertions, deletions, splice variants, gene copy numbers, or CpG islands within the genome for gene methylation studies, by performing additional bisulfite reactions. Advantages for this method over usual hybridization strategies are:

- Reduced mismatching due to intercession of the polymerase.
- Increased resistance to oligonucleotide failure sequences
- Tolerance of a greater range of hybridization conditions

#### Enzymatic Mutation Detection

Enzymatic Mutation Detection (EMD) is a streamlined and improved version of the original Enzymatic Cleavage of Mismatch (EMC) method. It is a fully homogeneous, rapid procedure with four steps: gene isolation, hybridization, detection and

analysis. It enables detection and localization of mismatched or unmatched nucleotides within heteroduplex DNA. EMD employs natural enzymes from cellular DNA recombination and repair systems that have been successfully selected and adapted for use in vitro. These enzymes recognize structural differences in DNA that arise from biochemical comparisons between normal and mutant DNA samples. This structural comparison can identify mutations much more effectively than the laborious process of direct determination of the entire DNA sequence of the gene. The entire procedure takes about 2 h and all the steps can be automated. Detection sensitivity approaches 98 %. Optimized microchip electrophoresis devices, combined with EMD methods, have been used to determination SNP sites in the p53 suppressor gene.

## DNA Sequencing

Most genetic disorders are caused by point mutations. Deletions are less frequent and may be overlooked by DNA mapping. It is difficult to find the location of a gene buried in the tangle of chromosomal DNA in the nucleus; sequencing of individual nucleotide bases may be required. DNA sequence analysis is a multistep process comprising sample preparation, generation of labeled fragments by sequencing reactions, electrophoretic separation of fragments, data acquisition, assembly into a finished sequence, and most importantly, functional interpretation. Sequencing is also used to determine protein sequences, but it is difficult to determine protein function from sequence. Sequencing is now automated. Sequencing technologies are described in a special report on this topic (Jain 2015b). Apart from their impact on hereditary neurologic diseases, high-throughput genome sequencing technologies will improve our understanding of sporadic neurologic diseases as well, particularly those with low-penetrant mutations in the gene for hereditary diseases or de novo mutations (Tsuji 2013).

### *Role of Sequencing in Personalized Medicine*

Among various technologies, sequencing will play an important role in the development of personalized medicine as shown in Fig. 2.1.

### *Whole Genome Sequencing and Personalized Medicine*

The role of sequencing in personalized medicine is supported by the latest effort in personal genome sequencing, Quake's genome sequence in 2010, which was undertaken as an integrated analysis of a complete human genome in a clinical

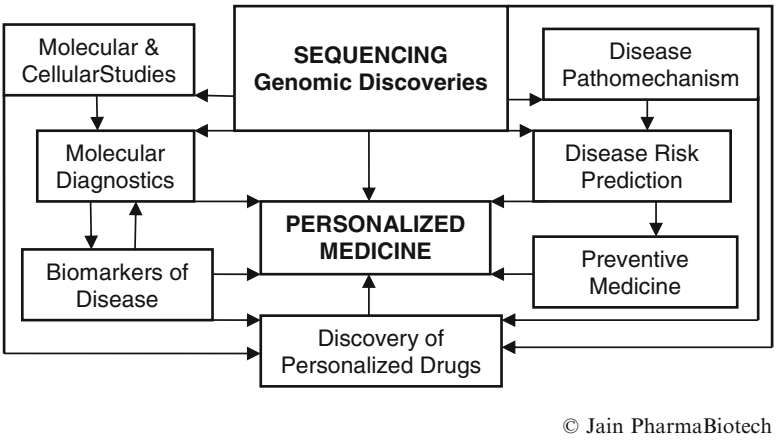


**Fig. 2.1** Role of sequencing in the development of personalized medicine

context on a person with a family history of vascular disease and early sudden death (Ashley et al. 2010). Sequencing was done with use of Heliscope single molecule sequencer (Helicos BioSciences) and reduced the cost to $50,000. Clinical assessment included analysis of this patient's full genome sequence, risk prediction for coronary artery disease, screening for causes of sudden cardiac death, and genetic counselling. Genetic analysis included the development of novel methods for the integration of whole genome and clinical risk. Disease and risk analysis focused on prediction of genetic risk of variants associated with mendelian disease, recognized drug responses, and pathogenicity for novel variants. The authors queried disease-specific mutation databases and pharmacogenomics databases to identify genes and mutations with known associations with disease and drug response. They estimated post-test probabilities of disease by applying likelihood ratios derived from integration of multiple common variants to age-appropriate and sex-appropriate pre-test probabilities. They also accounted for gene-environment interactions and conditionally dependent risks. Analysis of 2.6 million SNPs and 752 CNVs showed increased genetic risk for myocardial infarction, type 2 diabetes, and some cancers. Rare variants were discovered in three genes that are clinically associated with sudden cardiac death-TMEM43, DSP, and MYBPC3. A variant in LPA was consistent with a family history of coronary artery disease. The subject had a heterozygous null mutation in CYP2C19 suggesting probable clopidogrel resistance, several variants associated with a positive response to lipid-lowering therapy, and variants in CYP4F2 and VKORC1 that suggest he might have a low initial dosing requirement for warfarin. Many variants of uncertain importance were reported. Although challenges remain, these results suggest that whole-genome sequencing can yield useful and clinically relevant information for individual patients.

### *RNA Sequencing*

With the recognition of importance of RNA metabolism for brain function, as well as malfunction, there is an interest in understanding post-transcriptional gene regulation through many new and recently discovered mechanisms. Earlier transcriptomics studies were mostly based on hybridization-based microarray technologies and offered a limited ability to fully catalogue and quantify the diverse RNA molecules that are expressed from genomes over wide ranges of levels. Introduction of high-throughput NGS technologies have revolutionized transcriptomics by enabling RNA analysis through cDNA sequencing at massive scale (RNA-seq). This development has overcome several challenges posed by microarray technologies, including the limited dynamic range of detection (Ozsolak and Milos 2011). NGS platforms used for RNA-seq are commercially available from several companies, whereas new technologies are in development by others.

RNA-seq is a powerful tool for studying the effect of the transcriptome on phenotypes such as disease susceptibility, cancer progression and response to pharmaceuticals. Applications include the following:

- Transcript identification: mapping results reveal the identity of transcripts present in a sample, with ability to detect rare transcripts by increasing sequencing depth.
- Splice variant analysis: relative expression of exons across a single transcript can elucidate the presence of splice variants.
- Differential expression: differential expression levels of two transcripts in a single sample or of a single transcript in two disparate samples can be ascertained from relative sequencing depths.
- RNA measurements for clinical diagnostics, e.g. analysis of circulating extracellular nucleic acid and cells, such as fetal RNA. By enabling earlier diagnosis, disease recurrence or mutational status, this will help realization of the full potential of genomic information and its growing impact on the personalization of healthcare.

### *Whole Exome Sequencing and Personalized Disease Risk*

NGS is commonly used for researching the causes of genetic disorders. However, its usefulness in clinical practice for medical diagnosis is in early development. Replacing traditional methods for genetic testing of inheritable disorders with NGS will reduce the cost of genetic testing and increase the information available for the patients. NGS will become an invaluable resource for the patient and physicians, especially if the sequencing information is stored properly and reanalyzed as bioinformatics tools and annotations improve. NGS is still at the early stages of development, and it is full of false-positive and -negative results and requires infrastructure and specialized personnel to properly analyze the results.

A publication has demonstrated the value of NGS for genetic risk assessment and evaluated the limitations and barriers for the adoption of this technology into medical practice by performing whole exome sequencing (WES) in volunteers, and for each volunteer, they requested personal medical histories, constructed a three-generation pedigree, and required their participation in a comprehensive educational program (Gonzalez-Garay et al. 2013). This study explained the authors' experience with an adult population along with their bioinformatics analysis and clinical decisions to assure that genetic diagnostics were accurate to detect carrier status and serious medical conditions in volunteers. Furthermore, by incorporating family histories into their genetic analyses, they identified additional heritable diseases. Traditional genetic counseling and disease education were provided in verbal and written reports to all volunteers. This report demonstrates that when genome results are carefully interpreted and integrated with an individual's medical records and pedigree data, NGS is a valuable diagnostic tool for genetic disease risk. Limitations of this approach pointed put by the authors are:

- Bioinformatics focused on the practical extraction of medical relevant/actionable data are a challenge.
- Heavy reliance on HGMD alleles for "need to know" information by patients is flawed in three ways: (i) databases contain errors; (ii) highly validated disease databases are scattered, private, and limited; and (iii) the future will provide more disease risk alleles by sequencing than by patient reports in the literature.
- The current limitation for interpretation of a genome is not the quality of the data of the coverage of the genome but the Human Variome Project (http://www.humanvariomeproject) along with Beijing Genomics Institute is proposing to create a highly validated disease allele database to remedy this.
- The delivery of the genome risk information will need to be carried out by physicians and counselors skilled in medicine, genetics, and education/counseling. These experts will need to integrate into medical care as well as has been done for newborn screening, prenatal diagnosis, and newborn genetic disease diagnosis.

New technological advances such as structure-based prediction of protein–protein interactions on a genome wide scale, 3D structure of protein active and contact sites, high throughput functional assays of damaging alleles, and new approaches that combine analytes, metabolomics and genetic information from a single individual are just a few examples of the new technologies that will help us to generate better interpretation of genomic data. The approach of adult screening is in its early phase but appears very promising. Genomic study of adults deserves intensified effort to determine if "need to know" genome information can improved quality of health for the aging population.

### *Personal Genome Project*

Achieving personalized medicine will require extensive research on highly re-identifiable, integrated datasets of genomic and health information. A Personal Genome Project (PGP) was launched as a sequel of the Human Genome project and volunteers were recruited to make their own genomic and phenomic data available. Participants in the PGP choose to forgo privacy via institutional review board-approved "open consent" process. These resources were planned to include full (46-chromosome) genome sequences, digital medical records and other medical information that would become a part of personal health profile. It also includes comprehensive data about RNA and protein, body and facial measurements and imaging such as MRI. Human cell lines representing each subject are deposited in a repository at the National Institute of Genome Medical Sciences. Details of PGP can be found at the following web site: http://arep.med.harvard.edu/PGP/.

The findings after enrollment of more than 1,800 participants, including WGS of 10 pilot participant genomes (the PGP-10), have been published (Ball et al. 2012). The Genome-Environment-Trait Evidence (GET-Evidence) system, which automatically processes genomes and prioritizes both published and novel variants for interpretation, was introduced. In the process of reviewing the presumed healthy PGP-10 genomes, the authors found numerous literature references implying serious disease. Although it is sometimes impossible to rule out a late-onset effect, stringent evidence requirements can address the high rate of incidental findings. To that end the team developed a peer production system for recording and organizing variant evaluations according to standard evidence guidelines, creating a public forum for reaching consensus on interpretation of clinically relevant variants. Genome analysis becomes a two-step process: using a prioritized list to record variant evaluations, then automatically sorting reviewed variants using these annotations. Genome data, health and trait information, participant samples, and variant interpretations are all shared in the public domain. There is an open invitation to others to review the results using participant samples and contribute to interpretations. This public resource and methods are offered to further personalized medical research. In the ongoing project, the organizers hope to enroll 100,000 participants.

## Biochips and Microarrays

Biochip is a broad term indicating the use of microchip technology in molecular biology and can be defined as arrays of selected biomolecules immobilized on a surface. This technology has been described in more detail elsewhere (Jain 2015a). DNA microarray is a rapid method of sequencing and analyzing genes. An array is an orderly arrangement of samples. The sample spot sizes in microarray are usually <200 μm in diameter. It is comprised of DNA probes formatted on a biochip plus the instruments needed to handle samples (automated robotics), read the reporter molecules (scanners) and analyze the data (bioinformatic tools).

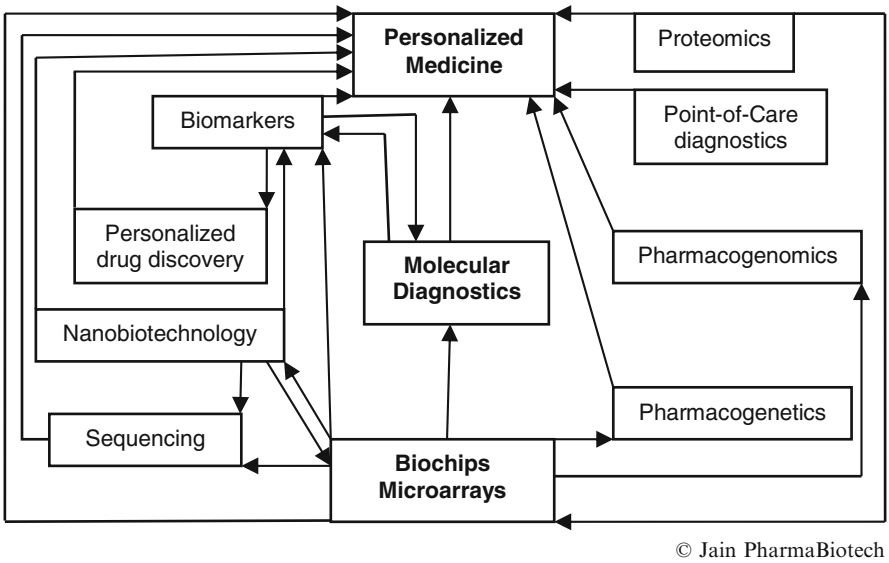


**Fig. 2.2** Role of biochip/microarray technology in personalized medicine

## *Role of Biochip/Microarray Technology in Personalized Medicine*

Microarray technology not only helps to make sense of the vast amount of genomic information but also enables its application to the patient by early detection of disease and prediction of drugs response in individuals. Although some problems of standardization and integration with electronic records remain, microarrays are promising for efficient, cost-effective, and personalized approaches to human health care. Role of biochip/microarray technology in personalized medicine is shown in Fig. 2.2.

## *Applications of Biochip/Microarray Technology in Personalized Medicine*

Selected applications of biochip technology relevant to this report are listed in Table 2.2.

Microarrays allow scientists to look at very subtle changes in many genes at one time. They provide a snapshot of what genes are expressed or active, in normal and diseased cells. When normal cells or tissues are compared to those known to be diseased, patterns of gene expression can emerge, enabling scientists to classify the

**Table 2.2** Applications of biochip technology relevant to personalized medicine

| |
|---|
| **Rapid DNA sequencing** |
| **Drug discovery and development** |
| High-throughput drug screening |
| Design and stratification of clinical trials |
| **Drug safety: applications in pharmacogenetics** |
| Toxicogenomics |
| Clinical drug safety |
| **Molecular diagnostics** |
| Genetic screening |
| Detection of mutations |
| Inherited disorders |
| Identification of pathogens and resistance in infections |
| Molecular oncology |
| Cancer prognosis |
| Cancer diagnosis |
| **Pharmacogenomics** |
| Gene identification |
| Genetic mapping |
| Gene expression profiling |
| Detection of single nucleotide polymorphisms (SNPs) |
| For storage of the patient's genomic information |
| **Integration of diagnosis and therapeutics** |



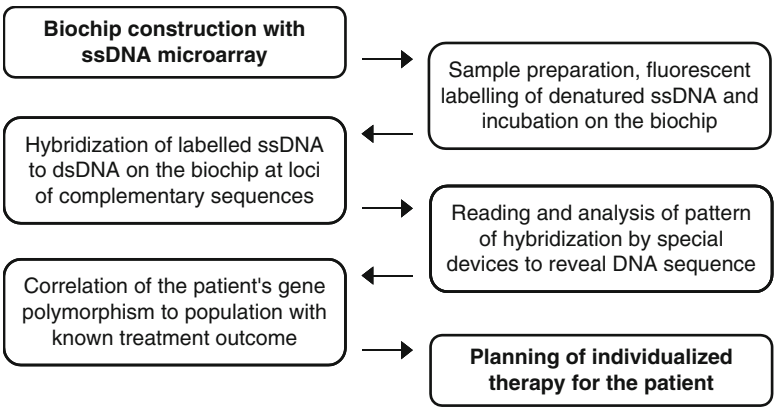


**Fig. 2.3** Application of biochips/microarrays in personalized therapy

severity of the disease and to identify the genes that can be targeted for therapy. This is how microarrays can be used to develop personalized medical treatments. Figure 2.3 shows development of biochips for pharmacogenomics and SNP genotyping to personalize medicine.

## *Biochip Technologies*

Numerous biochip technologies are available for clinical applications. Some examples that are relevant to personalized medicine are described briefly in the following text.

### PCR on a Chip

PCR is now offered on a chip. One example is VereChip™, a silicon chip that integrates an ultra-fast miniaturized PCR reactor for amplification of nucleic acids and a customizable microarray. It allows users to apply the full benefits of molecular testing in real-world conditions, at a fraction of the time, cost and complexity needed to operate common lab equipment. A compact optical reader captures and analyzes the microarray in a few seconds. The optimal optics settings are automatically selected and this makes it particularly suitable for minimally-trained personnel and point-of-care (POC) applications. The software can provide a highly detailed microarray analysis report for expert users in a central lab or a simple diagnostic report for basic users in a POC setting. An example of application is VereFlu™ for diagnosis of human and swine influenza.

A fast PCR biochip technology system can identify infectious disease strains in <15 min when using protein arrays and in <2 h when using nucleic acid arrays. The system can be used in hospitals and other laboratories as well as in the field. Each biochip has hundreds to thousands of gel drops, each ~100 μm in diameter. A segment of a DNA strand, protein, peptide or antibody is inserted into each drop, tailoring it to recognize a specific biological agent or biochemical signature. These drops are in known positions so when a sample reacts, the reaction position can be detected, identifying the sample. A sample to be tested is applied to a biochip, which is then put in a reader and scanned using patented side illumination laser technology to detect reaction sites. Automated algorithms determine the agents present in the sample. Techniques are being refined to shorten sample preparation time to ~10 min and increase system sensitivity, enabling full analysis to be done in <1 h for nucleic acid arrays.

Methods for the simultaneous analysis of multiple genes are needed and microarrays are an ideal platform for such analysis because their miniature size enables one to arrange up to hundreds or thousands of biological probes in a relatively small space with minute sample volume. However, the overall sensitivity of microarray detection technology is relatively low. Use of PCR as a powerful tool for multitarget analysis has two limitations: (1) identifying solution-phase multiplex PCR amplicons typically requires a secondary method for size separation or sequence verification prior to analysis and data interpretation; and (2) multiplex PCR is restricted in the number of targets that can be reliably amplified simultaneously, because of uncontrollable primer–primer interactions.

One solution for increasing the number of different targets that are amplified in a single PCR reaction is the spatial separation of different primer pairs. Microarrays are ideally suited for this task: tethering each pair of primers to a discrete spot on a surface directs the amplification of different targets in a number of non-overlapping micro-surroundings. Given the miniature dimensions of microarrays, highly multiplexed amplification would likewise occur in a homogenous, minimal volume and avoid the split assay. In multiplex microarray-enhanced PCR (MME-PCR), a highly sensitive method for DNA analysis, two amplification strategies are carried out simultaneously: on or within gel elements, and in bulk solution over the gel element array.

A universal DNA microarray combining PCR and ligase detection reaction (LDR) exploits full use of the sensitivity that the ligase detection reaction can provide, while maintaining a rapid read out on a universal microarray. It becomes fully programmable by uncoupling the mutation detection step from array hybridization. Main features of this method are:

- After hybridization of a discriminating probe and a common probe to the target sequence, ligation occurs only if there is perfect complementarity between the two probes and the template.
- The reaction is thermally cycled, generating single-stranded DNA fragments bearing a 5′ Cy3 fluorescent moiety and a 3′ czip code sequence.
- The cycling enables a more common probe (and the corresponding czip code) to ligate to the discriminating probe, given a fixed amount of PCR target.
- The LDR product is hybridized to a universal microarray, where unique zip code sequences have been spotted.

PCR/LDR/universal DNA microarray is 50-fold more sensitive and 10-fold more rapid than conventional hybridization-only arrays. It is a promising technology to help drive the transition from the current paradigms of clinical decision making to the new era of personalized medicine.

### Gene Profiling Array

A Gene Profiling Array (Affymetrix) is made using spatially patterned, light-directed combinatorial chemical synthesis and contain up thousands of different oligonucleotides on a small glass surface. In this approach sequence information is used directly to design high-density, 2D arrays of synthetic oligonucleotides, which are used for quantitative and highly parallel measurements of gene expression, to discover polymorphic loci and to detect the presence of thousands of alternative alleles. The latest product, Affymetrix® Gene Profiling Array cGMP U133 P2, gives the protein-coding content of the human genome on a single piece the size of a fingernail.

### Arrayit® H25K

Arrayit® H25K is the only genome microarray based on the completely sequenced human genome. It contains a fully annotated set of 25,509 human gene sequences and 795 controls. H25K is a multi-purpose long oligonucleotide microarray that allows karyotyping, gene expression profiling, chromatin structure analysis, and protein-DNA interaction studies on a genomic scale. Its glass substrate slide format is fully compatible with every major microarray scanner brand including the Arrayit InnoScan and SpotLight Scanner series.

### AmpliChip CYP450

AmpliChip CYP450 (Roche Molecular Diagnostics) is combined with the GeneChip 3000Dx microarray system of Affymetrix. It was cleared by the regulatory authorities for marketing in the US and the EU as an IVD test in 2004. The microarray chip (also referred to as "probe microarray") contains millions of tiny DNA molecules and the test is performed using DNA that is extracted from a patient's blood. DNA sequence is determined based on the sequence of the probe molecule to which the DNA is most similar. AmpliChip CYP450 contains >15,000 different oligonucleotide probes to analyze both the sense and the antisense strands of an amplified target DNA sample (Jain 2005).

By multiplexing long PCR reactions that amplify the promoter and coding region of CYP2D6, exons 4 and 5 of CYP2C19, as well as a 3.5-kb CYP2D6 gene deletion or CYP2D6 gene duplication-specific reactions, virtually all known polymorphisms and alleles of CYP2D6, and the two most frequent for CYP2C19, can be detected simultaneously. AmpliChip CYP450 comprehensively covers gene variations that play a role in the metabolism of ~25 % of all prescription drugs. The product characteristics are as follows:

- The chip has high built-in sensitivity for analyzing 29 polymorphisms and mutations for the 2D6 gene and 2 polymorphisms for the 2C19 gene, thereby increasing the probability of more accurately determining the genotype and phenotype. Accurately genotypes >99 % of the world's population.
- Analysis can normally be completed within a single 8 h shift.
- The software automatically determines genotype and predicted phenotype.
- Results are obtained and interpreted by computer analysis performed on a scan showing the pattern of hybridization of the patient sample to a series of probes (arrayed on the glass substrate) that are specifically complementary to either wild-type or mutant sequences.
- AmpliChip CYP450 test is intended to be an aid for physicians in individualizing treatment doses for patients on therapeutics metabolized through these genes.

DNA samples that represent nine different allelic variants including 2D6 gene deletions and duplications can be tested with the AmpliChip CYP450 microarray

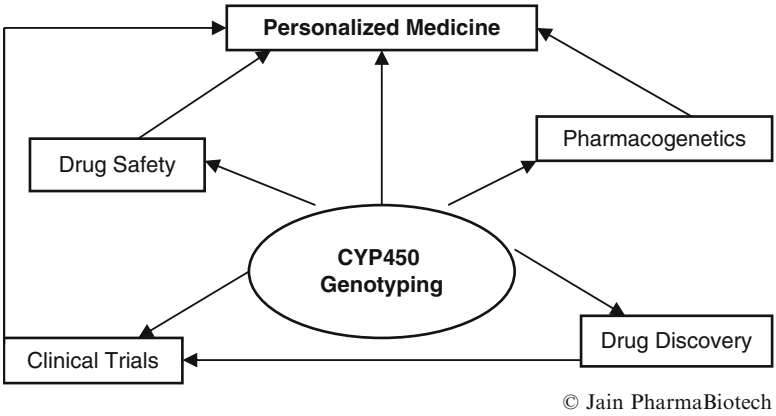


**Fig. 2.4** Role of CYP450 genotyping in development of personalized medicine

protocol with 100 % success rate. Small initial concentrations of DNA can be amplified and successfully genotyped on the AmpliChip CYP450 and a wide variety of sample types can be tested, including human whole blood, plasma and serum. Investigation of additional allelic variants on the AmpliChip CYP450 microarray is considered to be dependant on sample availability. The role of CYP450 genotyping in development of personalized medicine is shown in Fig. 2.4.

## *Standardizing the Microarrays*

Because researchers are using a lot of different methods and protocols in microarray experiments, it is difficult to compare their results with those from other laboratories. If microarrays are to be used effectively in the clinic to diagnose patients and design patient-tailored therapies, they will need to be standardized like any other clinical tests. Reproducibility between laboratories increases markedly when standardized protocols are implemented for RNA labeling, hybridization, microarray processing, data acquisition and data normalization. Reproducibility is highest when analysis was based on biological themes defined by enriched Gene Ontology categories. Use of commercially manufactured microarrays produced results that can be more easily replicated. Using microarrays made in-house by each lab gives less consistent results. However, microarray results can be comparable across different laboratories when a common platform and set of procedures are used. Improving and standardizing microarray experiments will also enable earlier detection of diseases and bring us one step closer to personalized medical treatment.

## *Optical Mapping*

Optical Mapping™ (OpGen Inc) involves the capture of multiple copies of whole genomes, as collections of long single DNA molecules isolated directly from cells without amplification or cloning, immobilized in dense arrays. Shotgun optical mapping approach can directly map genomic DNA by the random mapping of single molecules. Markers are scored simultaneously, in a single cost-effective manipulation, to produce high-resolution Optical Maps that can be used to characterize and compare genomes from any organism with no need for prior sequence information. This is a case of the right technology at the right time. Insertions and deletions (indels) appear to be more important than SNPs in accounting for sequence variation, evolutionary change and gene defects. Although Optical Mapping does detect SNPs, the system is primarily designed to identify genomic rearrangements, including indels, translocations and repetitive elements, in any genome. As attention shifts from SNPs to indels, Optical Mapping is perhaps the only system that can detect these events quickly, cheaply, and with high resolution, across entire genomes. Presence or absence of markers, and their distance apart, are scored to compare closely related genomes, to identify organisms and to detect genomic rearrangements such as indels. Optical Mapping has the following advantages over other methods for whole genome genetic analysis:

- The process involves only a single addition of reagents directly to native DNA, with no requirement for PCR, primers or probes, providing massively parallel, low cost marker analysis.
- It efficiently finds insertions, deletions, duplications, inversions, translocations, which are not readily detected by other methods such, SNP assays, and shotgun DNA sequencing.
- It can detect completely new and unsuspected genetic variation whereas probe-based systems are limited to measuring differences that have been found previously in other samples.
- It can survey entire human genomes for insertions/deletions, which account for a significantly greater proportion of genetic variation between closely-related genomes as compared to SNPs, and are a major cause of gene defects.

The advantage of Optical Mapping platform's freedom from dependence on sequence for de novo variant discovery has a downside to it, i.e. lower resolution than sequence-based approaches. The endpoints of any individual event can only be resolved to the nearest restriction site. This limitation is being addressed by developing alternative enzyme-based methods that increase marker density and add sequence information to mapped molecules. Algorithms are being developed to take advantage of the additional information for separating multiple genotypes at a single genomic locus. With further advances it will be possible to elucidate complex sequence-level events such as the somatic rearrangements that are a hallmark of cancer genomes.

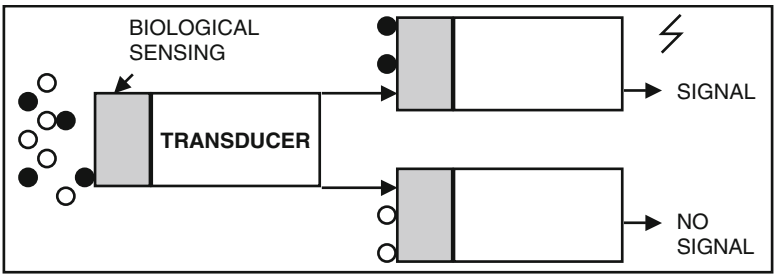


**Fig. 2.5** Basic principle of a biosensor. The compound of interest (*black circles*) in a mixture of substances specifically interacts with the biological sensing part of the sensor. The resulting biological signal is converted into a physical signal (e.g., electric or optical) by a transducer. Substances which are not capable of interacting with the biological component (*hollow circles*) will not produce any signal

## *Biosensor Technologies for Biochips*

Biosensors incorporate a biological sensing element that converts a change in an immediate environment to signals that can be processed. Biosensors have been implemented for a number of applications ranging from environmental pollutant detection to defense monitoring. Biosensors have two intriguing characteristics: (1) they have a naturally evolved selectivity to biological or biologically active analytes; and (2) biosensors have the capacity to respond to analytes in a physiologically relevant manner. Molecular biosensors are based on antibodies, enzymes, ion channels, or nucleic acids. In theory, nucleic acid analysis provides a higher degree of certainty than traditional antibody technologies because antibodies occasionally exhibit cross reactivity with antigens other than the analyte of interest. In practice, however, development of nucleic acid sensor systems has been hampered by the challenges presented in sample preparation. Nucleic acid isolation remains the rate-limiting step for all of the state-of-the-art molecular analyses.

Almost all analytical systems combine sensing (i.e. detection) and transducing components. The distinct feature of biosensors is that the two functions are coupled in a single physical entity. A biosensor's input is a specific biological event (e.g., binding of an antigen to an antibody). Its output is a measurable signal that corresponds to the input. Basic plan of a biosensor is shown in Fig. 2.5.

A biosensor's biological component provides specificity, the ability to selectively recognize one type of chemical or event. Its transducer confers sensitivity, the ability to transform the very low energy of the biological event into a measurable signal. In other words, a biosensor converts a biological event into an electrical signal. Biosensors would be useful in personalized medicine as feedback about status of biomarkers can guide therapeutics, e.g., glucose biosensors to monitor and guide insulin therapy according to individual requirements.

### DNA-Based Biosensors

DNA biosensors are being developed as alternatives to conventional DNA microarrays. These devices couple signal transduction directly to sequence recognition. Some of the most sensitive and functional technologies use fiber optics or electrochemical sensors in combination with DNA hybridization. Sensitivity of biosensors is being increased by incorporating nanotechnology to construct nanobiosensors. In a shift from sequence recognition by hybridization, some emerging single-molecule techniques read sequence composition using zero-mode waveguides or electrical impedance in nanoscale pores.

## *Protein Biochips*

Most of the biochips use nucleic acids as information molecules but protein chips are also proving to be useful. Profiling proteins will be invaluable, for example, in distinguishing the proteins of normal cells from early-stage cancer cells, and from malignant, metastatic cancer cells that are the real killers (Jain 2015d). In comparison with the DNA microarrays, the protein arrays, or protein chips, offer the distinct possibility of developing a rapid global analysis of the entire proteome leading to protein-based diagnostics and therapeutics.

Unfortunately, the methods for creating DNA chips cannot be applied to proteins. DNA is robust, whereas proteins are fragile. DNA can withstand extreme conditions and still retain its activity; proteins need a gentle environment or they will denature. Biochips enabled amplification of DNA so even very small amounts can be detected; no such techniques exist for proteins, but new biochip technologies are addressing this limitation.

Of all the applications of protein microarrays, molecular diagnostics is most clinically relevant and fits in with the trend in personalized medicine. These technologies have an advantage in diagnosis as different proteins such as antibodies, antigens, and enzymes can be immobilized within protein microchips. Miniaturized and highly parallel immunoassays greatly improve efficiency by increasing the amount of information acquired with single examination and reduce cost by decreasing reagent consumption.

### ProteinChip

ProteinChip (Bio-Rad) has a role in proteomics comparable to that of Genome Array in genomics. ProteinChip was the first complete tool for disease-focused protein biology. It is based on SELDI (surface-enhanced laser desorption/ionization) process, which has four parts as applied to patient samples:

- Patient sample of proteins is processed on the ProteinChip array.
- Enhance the "signal-to-noise" ratio by reducing chemical and biomolecular "noise" (i.e., achieve selective retention of target on the chip by washing away undesired materials).

- Read one or more of the target protein(s) retained by a rapid, sensitive, laser-induced process (SELDI) that provides direct information about the target (molecular weight).
- Process (characterize) the target protein(s) at any one or more locations within the addressable array directly in situ by engaging in one or more on-the-chip binding or modification reactions to characterize protein structure and function. Software produces map of proteins, revealing expression of marker protein with color change in the patient sample as compared to the control sample.

The ProteinChip system uses small arrays or plates with chemically or biologically treated surfaces to interact with proteins. Unknown proteins are affinity captured on treated surfaces, desorbed and ionized by laser excitation, and detected according to molecular weight. Known proteins are analyzed using on-chip functional assays. For example, chip surfaces can contain enzymes, receptor proteins or antibodies, enabling on-chip protein-protein interaction studies, ligand binding studies or immunoassays. With state-of-the-art ion optic and laser optic technologies, the ProteinChip System detects proteins ranging from small peptides of less than 1,000 Da up to proteins of 300 kDa or more and calculates the mass based on time-of-flight. The system includes ProteinChip arrays and reagents consumed in the process, the chip reader, software to analyze results and proprietary database to enable comparison between phenomic and genomic data. New ProteinChip Arrays have been packaged into a series of application-specific kits to enhance ease-of-use for the biologist performing protein analysis. ProteinChip Biomarker System enables clinical researchers to rapidly discover, characterize and validate predictive protein biomarkers and biomarker patterns in their own laboratories.

ProteinChip "benchtop" system and Tandem MS system have several advantages over the 2-D gel method. These include speed of detection (hours versus days), coverage of a broader region of the proteome, small sample requirement (1 ml or 500 cells) and combination of discovery and assay in a single system. An example of this is the discovery of prostate cancer biomarkers. With ProteinChip technology, it is possible to discriminate between benign prostatic hypertrophy with bound PSA (prostate-specific antigen) and cancer of the prostate with free PSA.

LumiCyte (a subsidiary of QIAGEN) has integrated SELDI with artificial intelligence-based data analysis capabilities and a powerful bioinformatics interrogation platform. Unlike other molecular mapping technologies, and protein biomarker detection assays designed to reveal disease, the SELDI BioChip molecular profiling platform enables both discovery and routine assays to be performed on the same BioChip in the same unit operation.

### Proteomic Pattern Analysis

Proteomic pattern analysis might ultimately be applied as a screening tool for cancer in high-risk and general populations. This also applies to autoimmune diseases, by screening sera of patients or high-risk individuals for the presence of

specific autoantibodies, using arrays of large numbers of recombinant proteins of known identity. Such arrays overcome the problems associated with variation of protein levels in conventional tissue extracts and hence improve reproducibility as a prerequisite for diagnostic use. High-throughput protein arrays have the potential to become diagnostic tools, eventually arriving at the doctor's office and as over-the-counter devices. However, techniques to enable efficient and highly parallel identification, measurement and analysis of proteins remain a bottleneck. A platform technology that makes collection and analysis of proteomic data as accessible as genomic data has yet to be developed. Sensitive and highly parallel technologies analogous to the nucleic acid biochip, for example, do not exist for protein analysis.

## New Developments in Protein Chips/Microarrays

The new and versatile protein array technology allows high-throughput screening for gene expression and molecular interactions. Protein arrays appear as new and versatile tools in functional genomics, enabling the translation of gene expression patterns of normal and diseased tissues into protein product catalogues. Protein function, such as enzyme activity, antibody specificity or other ligand–receptor interactions and binding of nucleic acids or small molecules can be analyzed on a whole-genome level. As the array technology develops, an ever-increasing variety of formats become available (e.g. nanoplates, patterned arrays, three-dimensional pads, flat-surface spot arrays or microfluidic chips), and proteins can be arrayed onto different surfaces (e.g. membrane filters, polystyrene film, glass, silane or gold). Various techniques are being developed for producing arrays, and robot-controlled, pin-based or inkjet printing heads are the preferred tools for manufacturing protein arrays. CCD cameras or laser scanners are used for signal detection; atomic force microscopy and mass spectrometry are upcoming options. The emerging future array systems will be used for high-throughput functional annotation of gene products, their involvements in molecular pathways, and their response to medical treatment and become the physician's indispensable diagnostics tools.

## Protein Biochips/Microarrays for Personalized Medicine

Protein biochips/microarrays are well-established tools for research and some products for in vitro diagnostics are available commercially. Profiling proteins on biochips will be useful for distinguishing the proteins of normal cells from early-stage cancer cells, and from malignant metastatic cancer cells. In comparison with the DNA microarrays, the protein microarrays/chips, offer the possibility of developing a rapid global analysis of the entire proteome leading to protein-based diagnostics and therapeutics. Of all the applications of protein microarrays, molecular diagnostics is most clinically relevant and would fit in with the coming trend in individualized treatment. These technologies have an advantage in diagnosis of some conditions.

For example, different proteins such as antibodies, antigens, and enzymes can be immobilized within protein biochips. Protein microarrays are reliable tools for detection of multiple biomarkers with only a minimal quantity of sample and have enormous potential in applications for personalized medicine (Yu et al. 2010).

## *Microfluidics*

Microfluidics is the special behavior of fluids flowing in channels the size of a human hair. Fluids in this environment show very different properties than in the macro world. This new field of technology was enabled by advances in microfabrication – the etching of silicon to create very small features. Microfluidics is one of the most important innovations of biochip technology. Microfluidics allows the reduction in size with a corresponding increase in the throughput of handling, processing and analyzing the sample. Other advantages of microfluidics include increased reaction rates, enhanced detection sensitivity and control of adverse events. Applications of microfluidics, in relation to molecular diagnostics, include the following:

- Genomic analyses
- Protein analysis
- Gene expression and differential display analysis

Several commercial microfluidic technologies are available and a few examples are described in the following text.

### Fish-on-Chip

Although interphase FISH is a sensitive diagnostic tool used for the detection of alterations in the genome on cell-by-cell basis, the cost-per-test and the technical complexity of current FISH protocols have limited its widespread utilization in clinical settings. To address this situation, a microchip-based FISH protocol has been devised and coupled with a novel method to immobilize peripheral blood mononuclear cells inside microfluidic channels (Sieben et al. 2007). These first on-chip implementations of FISH allow several chromosomal abnormalities associated with multiple myeloma to be detected with a tenfold higher throughput and one tenth the reagent consumption of the traditional slide-based method. Moreover, the chip test is performed within hours whereas the conventional protocol required days. In addition, two on-chip methods to enhance the hybridization aspects of FISH have been examined: mechanical and electrokinetic pumping. Similar agitation methods have led to significant improvements in hybridization efficiency with DNA microarray work, but with this cell-based method the benefits were moderate.

FISH-on-chip technology has potential clinical applications for cost-effective screening of cancer patients. The rapid detection of chromosomal mutations will increase a physician's ability to personalize treatment strategies to target individual cancers.

## Lab-on-a-Chip

A number of lab-on-a-chip devices have been constructed. An important limiting factor has been the difficulty of establishing molecular assays suitable for microfabricated formats. The assays should be capable of monitoring a wide range of molecules, including genomic DNA, RNA and proteins with secondary modifications and interaction partners, and they must exhibit excellent sensitivity and specificity (Melin et al. 2008). Incorporation of new molecular tools may provide opportunities for lab-on-a-chip devices at the POC.

The power of the lab-on-a-chip concept lies primarily in its ability to detect and manipulate at the cellular and molecular level with sufficiently high throughputs. With careful design and scaling considerations, molecular and cellular detectors (or biosensors) facilitated by controlled microfluidic separation, purification, sorting, and mixing operations are more sensitive and specific. Three levels of detection have been described (Lee 2009):

1. For DNA detection, a droplet microfluidic platform enables rapid and homogenous mixing in confined picoliter volumes for molecular hybridization fluorescence images
2. For proteins, an acoustic cavity mixer enables an order of magnitude increase in speed of detection
3. A novel microfluidic device based on dielectrophoresis enables the detection and sorting of biological cells based on their dielectric properties.

## LabChip

Lab-on-a-chip (PerkinElmer's LabChip), a miniaturized and integrated liquid handling and biochemical-processing device, is used for computer-aided analytical laboratory procedures that can be performed automatically in seconds. This as well as the Agilent 2100 bioanalyzer is being developed in collaboration with Agilent Technologies to integrate time-consuming and costly laboratory experiments onto a miniature chip. The applications menu for the Agilent 2100 Bioanalyzer includes nucleic acid analyses and protein assays – separation, sizing, quantifying and identifying what is in a sample of DNA, RNA, or proteins extracted from cells. PerkinElmer's genotyping system is designed to integrate each stage of the complete experiment in a volume of 1 nanoliter, a scale 10,000 to 100,000-fold smaller than currently used technology.

### LabCD

The LabCD (TECAN) is a consumable compact disc with micro-scale fluid paths, reaction chambers and valves. Fluid is moved along these pathways by capillary action and centrifugal forces generated by disc rotation, allowing the processing of many different assay types. The combination of informatics, bioassays and miniaturization are what make this "laboratory on a disc" truly revolutionary. The LabCD system is designed to meet the needs of the rapidly growing point-of-care testing market. Because of its distinct ability to combine assays utilizing different measurement methods, the LabCD can perform disease state panels from a single patient sample. In addition to providing immediate diagnostic results at the medical decision point, the LabCD automates data integration collected at various decentralized sites. The most important application of LabCD System is to screen for infectious diseases. In addition to providing automated DNA screening for infectious diseases, the LabCD will also be unique in its ability to test concurrently for different strains of the same virus from a single sample. For instance, physicians will be able to run tests for multiple strains of hepatitis all at the same time, instead of ordering them separately. The ability to identify the strain of a virus can have profound implications for clinical therapy.

## SNP Genotyping

High-resolution genome wide association studies using panels of 300,000 to 1 million SNPs aim to define genetic risk profiles of common diseases. These studies provide an opportunities to explore pathomechanism of human diseases that are unbiased by previous hypotheses or assumptions about the nature of genes that influence complex diseases. Many genetic variants identified as risk factors for diseases by such studies have been localized to previously unsuspected pathways, to genes without a known function.

In the absence of functional information about which polymorphisms are biologically significant, it is desirable to test the potential effect of all polymorphisms on drug response. Potential uses of SNP markers include drug discovery and prediction of adverse effects of drugs. Role of SNPs in personalized medicine is shown in Fig. 2.6.

SNP have the following relation to an individual's disease and drug response:

- SNPs are linked to disease susceptibility.
- SNPs are linked to drug response, e.g. insertions/deletions of ACE gene determine the response to beta blockers.
- SNPs can be used as markers to segregate individuals with different levels of response to treatment (beneficial or adverse) in clinical settings.

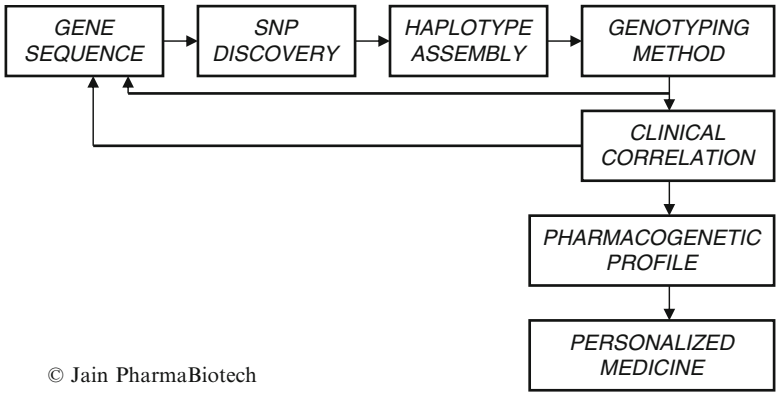


**Fig. 2.6** Role of SNPs in personalized medicine

The role of SNPs in clinical trials is:

- Genotyping is important in design and interpretation of clinical studies. Advantages of molecular genetic profiling in clinical studies are:
- It is a contribution to molecular definition of the disease
- Correlation of drug response to the genetic background of the patient
- Prediction of dose-response and adverse effects
- SNP mapping data can be used to pinpoint a common set of variant nucleotides shared by people who do not respond to a drug.

## *Genotyping and Haplotyping*

A genotype is the genetic constitution of an organism as defined by genetic and molecular analysis and covers the complete set of genes. Genotyping can be used for determination of relevant genetic variation in each of the two parental chromosomes in an individual.

Haplotypes are gene versions that represent the genetic variations as they occur on each pair of chromosome in an individual. This term has been redefined as a genetic bar code with each line representing a SNP. Gene-based haplotypes are comprised of a sequence of nucleotides (~25,000) that occur at SNP positions on a single chromosome at the locus of a single gene. Haplotypes are the most precise markers possible for a given gene because they contain all the variations in a gene. Haplotypes contain more information than unorganized SNPs and for practical purposes one has to deal with a dozen or fewer haplotypes for each gene. Thus, fewer patients are needed to detect statistically significant correlation to drug response than if SNP genotyping is used alone. This forms the basis of developing personalized or individualized therapy.

Haplotyping is an alternative approach to SNP genotyping. Haplotyping information makes it possible to highlight the structure of the genome, notably through haploblocks which correspond to segments of chromosomes unlikely to undergo a crossing-over event. Haplotyping is a way of characterizing combinations of SNPs that might influence response and is considered to be a more accurate measure of phenotypic variation. However, SNP-based tests have greater power when the number of causative SNPs (a subset of the total set of SNPs) is smaller than the total number of haplotypes. One limitation of haplotyping is that haplotypes need to be determined for each individual, as SNPs detected from a pool of DNA from a number of individuals cannot yield haplotypes.

Until whole-genome sequencing of individual patients becomes feasible clinically, the identification of SNPs and haplotypes will prove instrumental in efforts to use genomic medicine to individualize health care. When an extensive inventory of genome-wide SNP scans has been assembled across diverse population samples, maps using SNP and/or haplotypes will dictate that it will not be necessary to identify the precise genes involved in determining therapeutic efficacy or an adverse reaction. Linkage disequilibrium (LD) methods can provide robust statistical correlations between a patients response/risk index for a given drug class and a specific LD-SNP/haplotype profile.

Candidate gene-based haplotype approach has been applied to the pharmacogenetics of drug response and adverse events. Clinical trials using haplotyped individuals were the first genetically personalized medical treatments.

### Haplotyping for Whole Genome Sequencing

Despite considerable advances in whole-genome sequencing (WGS) in recent years haplotype information was still inadequate from whole genome sequencing. Only two genomes were completely haplotyped: the reference human genome and Craig Venter's genome, both of which relied on Sanger sequencing and clone mapping to resolve the haplotypes, which is a labor-intensive and costly process. Although the newer sequencing technologies enabled cost reductions and higher throughput, the shorter reads are not amenable to obtaining haplotype information, which will be critical in the fields of personalized medicine and population genetics. Disease risk prediction is difficult without haplotype information. Now two different but complimentary methods have been used to haplotype whole genomes; (1) combining NGS with large insert cloning to achieve a sequenced genome with haplotype information; and (2) using microfluidics technology in combination with genotyping to obtain haplotype information at the single-cell level.

The first method was used to determine the haplotype-resolved genome of a South Asian individual (Kitzman et al. 2011). A single fosmid library was split into a modest number of pools, each providing ~3 % physical coverage of the diploid genome. Sequencing of each pool yielded reads overwhelmingly derived from only one homologous chromosome at any given location. These data were combined with whole-genome shotgun sequence to directly phase 94 % of ascertained

heterozygous SNPs into long haplotype blocks. This method also facilitates the analysis of structural variation, for example, to anchor novel insertions to specific locations and haplotypes.

The second method used a microfluidic device capable of separating and amplifying homologous copies of each chromosome from a single human metaphase cell (Fan et al. 2011). SNP array analysis of amplified DNA enabled complete whole-genome, personal haplotyping of four individuals, including a HapMap trio with European ancestry and an unrelated European individual. The phases of alleles were determined at ~99.8 % accuracy for up to ~96 % of all assayed SNPs. Several practical applications were demonstrated including direct observation of recombination events in a family trio, deterministic phasing of deletions in individuals and direct measurement of the HLA haplotypes of an individual. This approach has potential applications in personal genomics, single-cell genomics and statistical genetics.

A method has been described for rapid and cost-effective long-range haplotyping (Kaper et al. 2013). Genomic DNA is diluted and distributed into multiple aliquots such that each aliquot receives a fraction of a haploid copy. The DNA template in each aliquot is amplified by multiple displacement amplification, converted into barcoded sequencing libraries using Illumina's Nextera technology, and sequenced in multiplexed pools. To assess the performance of this method, two male genomic DNA samples were combined at equal ratios, resulting in a sample with diploid X chromosomes with known haplotypes. Pools of the multiplexed sequencing libraries were subjected to targeted pull-down of a 1-Mb contiguous region of the X-chromosome Duchenne muscular dystrophy (DMD) gene. The authors were able to phase the DMD region into two contiguous haplotype blocks with a mean length of 494 kb. The haplotypes showed 99 % agreement with the consensus base calls made by sequencing the individual DNAs. They subsequently used the strategy to haplotype two human genomes. Standard genomic sequencing to identify all heterozygous SNPs in the sample was combined with dilution-amplification-based sequencing data to resolve the phase of identified heterozygous SNPs. Using this procedure, they were able to phase >95 % of the heterozygous SNPs from the diploid sequence data. The N50 for a Yoruba male DNA was 702 kb whereas the N50 for a European female DNA was 358 kb. Therefore, this strategy is suitable for haplotyping of a set of targeted regions as well as of the entire genome. The method can be used by any investigator with access to a NGS instrument.

Statistically aided, long-read haplotyping (SLRH) is a rapid, accurate method that uses a statistical algorithm to take advantage of the partially phased information contained in long genomic fragments analyzed by short-read sequencing (Kuleshov et al. 2014). For a human sample, as little as 30 Gbp of additional sequencing data are needed to phase genotypes identified by 50× coverage WGS. Using SLRH, the approach involved phasing 99 % of single-nucleotide variants in three human genomes into long haplotype blocks 0.2–1 Mbp in length. The authors applied this method to determine allele-specific methylation patterns in a human genome and identify hundreds of differentially methylated regions that were previously unknown. SLRH should facilitate population-scale haplotyping of human genomes. Compared with existing dilution haplotyping methods, SLRH produces haplotypes

of equal or greater quality using substantially less sequencing effort. Tools that facilitate access to phase information will lay the foundation for further advances throughout genomics and contribute to the development of personalized medicine.

#### Predicting Drug Response with HapMap

A pharmacogenetic study in cardiovascular disease using a model based on HapMap revealed that haplotype constituted by allele Gly16 (G) at codon 16 and allele Glu27 (G) at codon 27 genotyped within the beta2AR candidate gene exhibits a different effect on heart rate curve than the rest of haplotypes (Lin et al. 2005). Parents with the diplotype consisting of two copies of haplotype GG are more sensitive in heart rate to increasing dosages of dobutamine than those with other haplotypes. This model provides a powerful tool for elucidating the genetic variants of drug response and ultimately designing personalized medications based on each patient's genetic constitution.

## *Technologies for SNP Analysis*

Technologies used for detection and analysis of SNPs are shown in Table 2.3. These are described in more detail elsewhere (Jain 2015a) but some are described briefly in the text following Table 2.3. Desirable characteristics of a genotyping technology are: (1) robust performance and accuracy across a variety of circumstances; (2) high-throughput performance; and (3) low cost. Sequencing offers the highest degree of specificity and selectivity. Restriction fragment length polymorphism (RFLP), TaqMan assays and DNA microarrays are also frequently used genotyping methods.

## *Biochip and Microarray-Based Detection of SNPs*

#### SNP Genotyping by MassARRAY

The starting point for SNPs analysis using the MassARRAY system (SEQUENOM) is genomic DNA that is easily accessed in a sample of biological material such as blood. A small amount of blood provides sufficient material to allow tens of thousands of SNPs to be analyzed in one individual. DNA is prepared by standard procedures that break open the blood cells, release the DNA and discard other material. Next, specific DNA regions, about 200 base pairs in length, are amplified by enzymatic reactions into multiple copies to produce more concentrated samples for easier analysis. The amplified fragments are then attached by one strand to a solid surface and the non-immobilized strands are removed by standard denaturation and washing. The remaining immobilized single strand then serves as a template for

**Table 2.3** Technologies for SNP analysis

| |
|---|
| Digital genetic analysis (DGA) |
| DNA chips and microarrays |
| DNA sequencing |
| Electrochemical DNA detection |
| **Hybridization assays** |
| Allele-specific oligomer hybridization |
| Array hybridization assays, e.g., MASDA (mutiplexed allele-specific diagnostic assay) |
| Hybridization with PNA probes |
| Mass spectrometry (MS) |
| Matrix assisted laser desorption ionization time of flight MS (MALDI-TOF MS) |
| Competitive oligonucleotide single base extension (COSBE) |
| Nanotechnology-based methods |
| PCR-based methods |
| PCR-CTPP (confronting two-pair primers) |
| Degenerate oligonucleotide primed (DOP)-PCR |
| TaqMan real-time PCR |
| Smart amplification process version 2 |
| Peptide nucelic acid (PNA) probes |
| Pyrosequencing |
| Restriction-fragment-length polymorphism (RFLP) |
| Zinc finger proteins |



automated enzymatic reactions that produce genotype specific diagnostic products. Very small quantities of these products, typically 5–10 nanoliter, are transferred with the SpectroJET nanoliter dispensing system onto the high density SpectroCHIP array for subsequent automated analysis with the SpectroREADER mass spectrometer. Because DNA is composed of four bases (A, T, G and C) and each has a unique mass, the mass of the resultant diagnostic product can be used to unambiguously identify the sample's genotype. The SpectroTYPER software then calculates, records, compares and reports the genotypes at the rate of 3 s/per sample.

There is a proof of concept of the use of pooled DNA as a means of efficiently screening SNPs and prioritizing them for further study. This approach reduces the final number of SNPs that undergo full, sample-by-sample genotyping as well as the quantity of DNA used overall. Genotyping of the individual samples shows that the average margin of error in frequency estimate is ~4 % when pools are used. These findings clearly demonstrate the potential of pooling techniques and their associated technologies as an initial screen in the search for genetic associations.

### BeadArray Technology

BeadArray technology (Illumina) combines fiber optic bundles and specially prepared beads that self-assemble into an array. Each fiber optic bundle contains thousands to millions of individual fibers depending on the diameter of the bundle.

In a separate process, biosensors are created by affixing a specific type of molecule to each of the billions of microscopic beads in a given batch. The particular molecules on a bead define that bead's function as a biosensor. Batches of beads coated with specific molecules are combined to form a pool specific to the type of intended array provides substantial levels of throughput for the detection of DNA sequences and genotyping.

### SNP-IT Primer-Extension Technology

SNP-IT primer-extension technology is a method of isolating the precise location of the site of a suspected SNP and utilizing the inherent accuracy of DNA polymerase to determine the presence or absence of the SNP. In order to conduct SNP-IT primer extension, a specially synthesized DNA primer is bound to the sample DNA to expose the DNA site of interest where a SNP may be present. DNA polymerase, a naturally occurring molecule that accurately and reliably inserts the appropriate complementary base to a chain of DNA, is then added to extend the DNA chain by one base at the suspected SNP location. Several conventional methods are then used, including fluorescence, optical density, electrophoresis and mass spectroscopy, to detect this single base extension. The result is a direct read-out method of detecting SNPs that creates a simple binary "bit" of genetic information representing the presence of a SNP in a DNA sample. Platforms that can use SNP-IT include mass spectrometry and microarrays.

### Use of NanoChip for Detection of SNPs

The NanoChip® Electronic Microarray (Savyon Diagnostic) is the only commercial product on the market that uses electronically enhanced hybridization of complementary DNA strands. In several research studies, the NanoChip has achieved 100 % accuracy in the detection of SNPs. This technology allows researchers to perform "multiplex" assays (the ability to run assays that determine the presence or absence of multiple genetic mutations at the same time and on the same chip). Savyon is developing technology to allow the on-chip amplification of DNA material directly on the NanoChip cartridge, which eliminates a time-consuming preparatory step and folds it into a single, simplified detection procedure.

### Electrochemical DNA Probes

DNA is electrochemically silent unless highly negative and positive potentials are applied, at which time, solvent decomposition occurs and interferes with the signals from DNA. Several assays based on electrochemical DNA detection have been used for SNP genotyping. Another approach for electrochemical detection of SNPs exploits a method that relied on charge transport through the $\pi$-stack of duplexes

(18–22) on gold electrodes by combining redox-active intercalators with exogenous electrocatalytic species. None of these methods is quite satisfactory, particularly for identifying SNPs of each individual patient.

A highly sensitive electrochemical detection of complementary DNAs (up to 43-mer) is based on hole transport with molecular-scale, "wire-like" DNA probes. The presence of a single-base mismatch in the DNA duplexes causes a dramatic decrease in the electrochemical response. This method has been applied to detect all of the possible transition and transversion SNPs and achieved "on-off"-type discrimination of fully complementary DNAs from their SNPs. Furthermore, naturally occurring polymorphisms, "hot spots" from the p53 gene, can clearly be distinguished from wild type by using this method. The sensitivity may increase to a sufficient level that enables direct pathogen detection. More, the reagent-free detection of unmodified target DNAs is suitable for applying this method to biochip-based genetic analysis.

### Laboratory Multiple Analyte Profile

Laboratory Multiple Analyte Profile (LabMap) technology has been developed by Luminex Corporation (Austin, TX). There are four core components of this technology:

1. The biomolecule of interest, which can be any analyte, including oligonucleotide DNA probes.
2. Fluorescently dyed microspheres that function as carriers of biomolecules.
3. Bench-top analyzer with lasers and optics that register individual events.
4. A high-speed digital signal processor to quickly manage the fluorescent output.

LabMap simultaneously measures all the analytes for any molecular relationship in one sample smaller than a single drop of blood. Advantages of this technology include the following:

- All-in-one reactions save on labor, reagents and consumables
- One instrument tests nucleic acids, immunoassay, enzymes, and receptor-ligands
- Rapid kinetics lowers incubation times.
- High throughput (20,000 microspheres per second) shortens analysis time
- No radioactive disposal
- Western blot capabilities without gels and human variability

### PCR-CTPP

PCR-CTPP (confronting two-pair primers for polymorphism) genotyping has been found to be suitable for genotyping in studies of genetic epidemiology involving hundreds of samples. In this method, CTPP is introduced to detect a SNP (base X or

Y). One primer for the X allele is set to include X′ at the 3′ end (antisense), where X′ is the antisense of X, with the counterpart sense primer upstream. For the Y allele, a sense primer including Y at the 3′ end is set, with the antisense primer downstream. One common band and one specific band for each allele are amplified, which allows genotyping directly by electrophoresis. This method is exemplified by application to the polymorphisms of beta-adrenoceptor 2 and interleukin 1B. It is simpler than RFLP (restriction fragment length polymorphism), which requires incubation with a restriction enzyme.

### TaqMan Real-Time PCR

TaqMan (Life Technologies/Thermo Fisher) is a real-time PCR method that enables one-step mutation detection. In PCR, forward and reverse primers hybridize to a specific sequence of the target DNA in order to amplify the target sequence. The TaqMan probe, with its bound fluorophore and quencher, hybridizes to a second target sequence within the amplified product. When the PCR product is further amplified in subsequent cycles, the AmpliTaq enzyme cleaves the TaqMan probe (5′ nuclease activity) so that it can continue to copy its target sequence.

The reporter dye and quencher dye are separated, resulting in increased fluorescence of the reporter. This process occurs in every amplification cycle and does not interfere with the exponential accumulation of product. Because release of the reporter dye is associated with the amplification of the specific gene DNA, the fluorescent signal is generated only if the gene sequence is present in the sample.

The TaqMan method can detect as few as five copies of target in a background of 500 ng of DNA. The risk of PCR contamination is eliminated with this technology because detection occurs within the amplification reaction, thus also eliminating postamplification analysis costs.

### Locked Nucleic Acid

Locked Nucleic Acids (LNA) is a novel oligonucleotide analogue containing a conformationally restricted nucleotide with a 2′-O, 4′-C-methylene bridge that induces unprecedented thermal affinities when mixed with complementary single stranded DNA and RNA. LNA combines the highest affinity ever reported for a DNA analog for complementary DNA and RNA with a superb ability to discriminate between correct and incorrect target sequences. This property is extremely important for diagnostic use. An example of application of LNA technology for personalized medicine is that it has been used successfully been to develop ELISA-assays that detect the Apolipoprotein B R3500Q mutation. This facilitates a rational screening of patients with cardiovascular disease for abnormalities in levels and metabolism of lipoproteins.

### Molecular Inversion Probe Based Assays

A molecular inversion probe (MIP) is an unmodified oligonucleotide (110–140 bases) that contains the following functional segments:

- Two sequences homologous to sites adjacent to a SNP of interest
- A unique tag sequence specific to each MIP that can be hybridized to an array
- Two sequences that anneal PCR primers common to all MIP probes

Using Lab-in-a-tube™ technology MIP enables multiplexed SNP scoring to levels of 10,000 reactions in parallel. MIP genotyping is particularly effective when genotyping >500 SNP's with >100 samples.

### Pyrosequencing

Pyrosequencing technology is based on DNA sequencing and is aimed at laboratories performing medium to high-throughput screening of characterized SNPs. Pyrosequencing is a sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light, which is proportional to incorporated nucleotides In contrast to some of the other methods discussed, pyrosequencing takes into account the DNA sequence flanking the SNP, which helps to reveal sequencing artifacts and so reduces the number of false positive results which are generated. Unlabeled sequencing primer is hybridized to the DNA target (PCR product) and a single labeled ddNTP is added to the reaction mixture containing the enzymes DNA polymerase, ATP sulfurylase, luciferase and apyrase in the presence of the substrates adenosine 5′ phosphosulfate (APS) and luciferin. If ddNTP is incorporated into the DNA strand, pyrophosphate (PPi) is released and reacts with APS to generate ATP in equimolar quantities to the amount of ddNTP introduced into the nascent strand. ATP drives the conversion of luciferin to oxyluciferin, which generates light as a by-product. The light is detected by a CCD camera and is registered as a single peak on the pyrogram. Any remaining ATP and unincorporated ddNTP is upgraded by the action of apyrase prior to addition of the second ddNTP. As the process continues, the DNA strand is extended and the nucleotide sequence is determined from the relative height and number of signal peaks in the pyrogram.

Pyrosequencing enables genotyping of 96 samples within 10 min with an accuracy of >99 %. It can also be used to determine allele frequencies of known SNPs in pooled populations of genomic DNA. Pyrosequencing technology offers a highly automated, rapid, and accurate method for identification of cytochrome P450 alleles, which is suitable for pharmacogenomic research, as well as for routine assessment of patient genotypes. In pyrosequencing, a single set of PCR and sequencing primers is used to coamplify and sequence a region in the CYP2D6 gene and the equivalent region in the CYP2D8P pseudogene with relative quantification between these fragments. The use of an internal CYP2D8P control as well as generation of a sequence context insures robustness and facilitates validation of the method.

### Smart Amplification Process Version 2

A rapid SNP detection system called smart amplification process version 2 (SMAP 2) has been reported (Mitani et al. 2007). Because DNA amplification only occurred with a perfect primer match, amplification alone was sufficient to identify the target allele. To achieve the requisite fidelity to support this claim, the authors used two new and complementary approaches to suppress exponential background DNA amplification that resulted from mispriming events. SMAP 2 is isothermal and achieved SNP detection from whole human blood in 30 min when performed with a new DNA polymerase that was cloned and isolated from Alicyclobacillus acidocaldarius (Aac pol). Furthermore, to assist the configuration of SMAP 2 assays, software specific for SMAP 2 primer design was developed. With these new tools, a high-precision and rapid DNA amplification technology has become available for pharmacogenomic research and molecular-diagnostics applications. A study has confirmed that PNA-SMAP2 combination has higher sensitivity and accuracy than traditional sequencing methods or PCR and is suitable for the clinical diagnosis of KRAS codon 12 mutations (Araki et al. 2010).

### Zinc Finger Proteins

Zinc finger DNA-binding proteins (ZFPs) are the dominant class of naturally occurring transcription factors in organisms from yeast to humans. ZFPs can effectively detect small variations in DNA sequences and therefore may be used to detect SNPs in clinical samples. ZFPs have the potential to eliminate the extensive manipulation of patient DNA samples, reducing the time and cost, and increasing the accuracy of diagnostic assays. Sangamo Biosciences intends to commercialize ZFPs for SNP detection and DNA diagnostics in conjunction with partners engaged in the development of SNP diagnostic technology or the manufacturing and marketing of clinical diagnostics.

### Mitochondrial SNPs

While autosomal nuclear DNA genes are confined to the nucleus, limited to two copies per cell, the mitochondrial DNA (mtDNA) genes are distributed throughout the cytoplasm and are present in numerous copies per cell. The mtDNA molecule is relatively small containing 16,569 nucleotide pairs. There is growing evidence that defects of mtDNA causes disease. Majority of these defects are due to point mutations or rearrangements of the mitochondrial genome, while others, such as mtDNA deletions, are autosomally-linked. More than 100 mutations of mtDNA been associated with a striking variety of multisystem as well as tissue-specific human diseases.

Mitochondrial SNPs (mtSNPs) constitute important data when trying to shed some light on human diseases. Amongst the several methods reported for SNP

genotyping, determining the restriction fragment length polymorphisms (RFLPs) is still one of the most convenient and cost-saving methods. Abnormalities in mitochondrial complex I, which is responsible for controlling mitochondrial function, have been implicated in a variety of diseases associated with mitochondrial dysfunction including schizophrenia. The NADH dehydrogenase Fe-S protein 1 (NDUFS1) is the largest subunit of complex I. Findings of a study suggest that NDUFS1 may confer susceptibility to schizophrenia in male subjects, acting as a causative factor for the severity of negative symptoms in schizophrenia (Zhu et al. 2014).

### Limitations of SNP in Genetic Testing

Genotyping for complex diseases may be insufficient to predict whether a person is at risk for a particular disease. One tries to associate SNPs with disease, but if no SNP in a certain gene predicts disease, further interest in the gene or protein or enzyme is lost. In some cases, the phenotype expressed by a gene provides a more accurate risk assessment. Genotypes of the paraoxonase (PON1) gene on chromosome 7 are associated with atherosclerosis or cardiovascular disease (CVD) but the genotype in these subjects does not always reflect the phenotype of patients with CVD. These results support the benefit of a "level crossing" approach that includes intervening phenotypes in the study of complexly inherited disease. Therefore, there is a need to look beyond SNP studies to understand genetic contributions to complex disease.

Another example is the search for a gene for phenylketonuria (PKU), where the investigators have looked for an association between individual restriction fragment-length polymorphisms (RFLPs), and PKU mutations. While haplotypes were clearly associated with disease, individual RFLP sites were not because they will cancel out if there are many mutations at a locus.

### Concluding Remarks on SNP Genotyping

Several methods are available for SNP genotyping. For 10 or fewer SNPs and sample numbers in the thousands, the current gold standard is TaqMan real-time PCR. MassARRAY system, a mass spectrometry-based platform, is suitable for high throughput and up to 1,000 SNPs. Pyrosequencing, a sequencing-by-synthesis method can be used for up to 100 SNPs. Affymetrix provides the densest coverage at the whole-genome level with its GeneChip Human Mapping 500 K Array Set and Affymetrix GeneChip® Scanner 3000 MegAllele, and enables the highest level of multiplexing that is commercially available as well as increase throughput with low capital investment. Illumina is supplementing its current 100 K chip with a 250 K chip. RFLP analysis is laborious and hit-and-miss as success depends on whether the restriction enzyme recognizes particular SNPs. It is relatively inexpensive, which makes it appropriate for a small number of SNPs and a small number of samples. New methods for SNP genotyping are being investigated. The presence of

a single base pair mismatch can be identified by the conductance of the molecule and can cause a change in the conductance of dsDNA by as much as an order of magnitude, depending on the specific details of the double helix and the SNP.

### Impact of SNPs on Personalized Medicine

Pharmacogenetic capabilities have markedly increased since the first SNP map from the SNP Consortium became available in 2001. SNP-mapping technologies now enable us to create a genetic profile of each individual that can be used to identify patterns of susceptibility genes for common diseases as well as genetic risk/efficacy factors that are related to the effects of drugs. Inter-individual variability in drug response, ranging from lack of efficacy to life-threatening adverse reactions is influenced by variation in genes that control the absorption, distribution, metabolism and excretion of drugs.

An example of how SNP genotyping may be applied in medicine is the evidence of association between an SNP in the TNFR (tumor necrosis factor receptor) II gene and rheumatoid arthritis. TNF is a powerful mediator of inflammation in rheumatoid arthritis. In vivo, its acute effects are limited by binding to TNFR, suggesting that TNFR genes could be important candidate risk factors, the strongest association being observed in patients with a family history of this disease. The TNFR2 polymorphism or other genetic variations in the TNF or related genes may be useful biomarkers for susceptibility to familial rheumatoid arthritis and response to treatment with TNF inhibitors.

## Detection of Copy Number Variations

Although the importance of CNVs in genome wide association studies (GWAS) is widely accepted, the optimal methods for identifying these variants are still under evaluation. Extensions of GWAS to CNV have already resulted in discoveries of both de novo and inherited CNVs associated with risk of common disease. CNVs in the human genome are detected with high-throughput scanning technologies, such as CGH and high-density SNP microarrays, or even relatively low-throughput techniques, such as quantitative PCR. A comprehensive view of CNVs in the HapMap DNA collection using high density 500 K Early Access SNP genotyping arrays has revealed >1,000 CNVs ranging in size from 1 kb to over 3 Mb. Although the arrays used most commonly for GWAS predominantly interrogate SNPs, CNV identification does not necessarily require the use of DNA probes centered on polymorphic nucleotides and may even be hindered by the dependence on a successful SNP genotyping assay. Non-polymorphic probes provide a robust approach for CNV identification, and the increasing precision of CNV boundary delineation should allow a more complete analysis of their genomic organization. Development of sequencing technologies has opened the door to novel methods for detecting CNVs

in the human genome, which can also be used for the reliable identification of large copy-variable regions. Problems with the methods include sequencing biases that lead certain regions of the genome to be over- or under- sampled, lowering their resolution and ability to accurately identify the exact breakpoints of the variants.

### *Digital Array for CNV Detection*

Most of these approaches are limited in resolution and can at best distinguish a twofold (or 50 %) difference in CNV. CNVs can be studied by using digital array, a nanofluidic biochip capable of accurately quantitating genes of interest in DNA samples. This technology is exquisitely sensitive and is capable of differentiating as little as a 15 % difference in CNV or 6–7 copies of a target gene. Analysis of DNA samples for their CYP2D6 copy numbers shows that the results are consistent with those obtained by conventional techniques. In a screening experiment with breast cancer and normal DNA samples, the ERBB2 gene was found to be amplified in about 35 % of breast cancer samples (Qin et al. 2008). Thus the use of the digital array enables accurate measurement of gene copy numbers and is of significant value in CNV studies.

### *CNVer Algorithm for CNV Detection*

An algorithm for CNV detection, called CNVer, supplements the depth-of-coverage with paired-end mapping information, where mate pairs mapping discordantly to the reference serve to indicate the presence of variation (Medvedev et al. 2010). It combines the information from high-throughput sequencing within a unified computational framework called the donor graph, enabling the mitigation of sequencing biases that cause uneven local coverage and accurately predict CNVs. CNVer was used to detect 4879 CNVs in genome of a Yoruban individual. Most of the calls (77 %) coincide with previously known variants within the Database of Genomic Variants, while 81 % of deletion copy number variants previously known for this individual coincide with one of our loss calls. Furthermore, it was demonstrated that CNVer can reconstruct the absolute copy counts of segments of the donor genome and evaluate the feasibility of using CNVer with low coverage datasets.

### *CNVnator for Discovery of CNVs and Genotyping*

CNVnator has been developed for CNV discovery and genotyping from read-depth analysis of personal genome sequencing (Abyzov et al. 2011). This method is based on combining the established mean-shift approach with additional refinements to

broaden the range of discovered CNVs. CNVnator was calibrated by using the extensive validation performed by the 1000 Genomes Project. Because of this, CNVnator could be used for CNV discovery and genotyping in a population and characterization of atypical CNVs, such as de novo and multi-allelic events. Overall, CNVnator has high sensitivity, low false-discovery rate, high genotyping accuracy, and high resolution in breakpoint discovery. Furthermore, CNVnator is complementary in a straightforward way to split-read and read-pair approaches. It misses CNVs created by retrotransposable elements, but more than half of the validated CNVs that it identifies are not detected by split-read or read-pair. By genotyping CNVs in the CEPH, Yoruba, and Chinese-Japanese populations, it was estimated that at least 11 % of all CNV loci involve complex, multi-allelic events, a considerably higher estimate than reported earlier. Moreover, among these events, the authors observed cases with allele distribution strongly deviating from Hardy-Weinberg equilibrium, possibly implying selection on certain complex loci. Finally, by combining discovery and genotyping, they identified six potential de novo CNVs in two family trios.

## Study of Rare Variants in Pinpointing Disease-Causing Genes

Genome-wide association studies (GWAS) use gene chips in automated systems that analyze about 500,000 to 1 million sites where SNPs tend to occur. In using these SNP chips over the past decade in comparing DNA samples between healthy subjects and patients, scientists have identified thousands of SNPs that associate with common complex diseases. However, SNPs investigated by the gene chips do not themselves cause a disease, but instead serve as a marker linked to the actual causal mutations that may reside in a nearby region. After a GWAS finds SNPs linked to a disease, researchers then perform a "fine-mapping" study by additional genotyping, i.e. sequencing of the gene regions near the SNP signal, to uncover an altered gene that harbors a mutation responsible for the disease.

GWAS have been successful in identifying disease susceptibility loci, but pinpointing of the causal variants in subsequent fine-mapping studies remains a challenge. A conventional fine-mapping effort starts by sequencing dozens of randomly selected samples at susceptibility loci to discover candidate variants, which are then placed on custom arrays and algorithms are used to find the causal variants. A new study challenges the prevailing view that common diseases are usually caused by common gene variants (mutations) but the culprits may be numerous rare variants, located in DNA sequences farther away from the original "hot spots" than scientists have been accustomed to look (Wang et al. 2010). The authors propose that one or several rare or low-frequency causal variants can hitchhike the same common tag SNP, so causal variants may not be easily unveiled by conventional efforts. They demonstrated that the true effect size and proportion of variance is explained by a collection of rare causal variants, which can be underestimated by a common tag SNP, thereby accounting for some of the "missing heritability" in GWAS.

Sequencing DNA in subset of patients most likely to carry causative mutations leads to identification of more actual mutations. This refined technique may identify individuals more likely to have mutations in causal genes. By applying their methods to real DNA samples from patients with genetic hearing loss, the researchers' approach helped them to select from GWAS data a subset of cases for sequencing analysis that were most likely to carry causative mutations. Sequencing the DNA in this subset, the study team found that the majority of those patients carried an actual mutation known to cause hearing loss. This approach will facilitate personalized medicine, in which treatment will be tailored to an individual's genetic profile. Identifying causal variants in disease genes provides an opportunity to develop drugs to rectify the biological consequences of these mutated genes.

## Application of Proteomics in Molecular Diagnosis

Discovery of the genetic sequence encoding a protein by nucleic acid technologies is not sufficient to predict the size or biological nature of a protein. Studies at the messenger RNA (mRNA) level can assess the expression profiles of transcripts but these analyses measure only the relative amount of an mRNA encoding a protein and not the actual amount of protein in a tissue. To address this area, several protein-based analysis technologies have been developed.

Proteomics investigations endeavor to provide a global understanding of gene product synthesis rate, degradation rate, functional competence, posttranslational modification, subcellular distribution and physical interactions with other cell components. Usual sequence of events in proteomics is as follows: samples → protein separation → gel analysis → differential protein expression → sequence analysis. Bioinformatic systems integrate clinical data, robotics and protein identification into an automated process.

There is no protein analog of PCR and most of the work in the past has been manual. 2D GE has been the key technology for protein expression and mass spectrometry is the method of choice for connecting the genome and proteome worlds.

Proteomic technologies are considered to be a distinct group within molecular diagnostics and should not be confused with immunoassays although some proteomic technologies are antibody-based. Proteomics will facilitate mass screening at the protein level to supplement the genetic screening and fill a gap in molecular medicine. Proteomic data can provide clinical biomarkers for monitoring patient progress (see Chap. 3).

### *Proteomic Technologies*

Technologies with the greatest potential are 2D PAGE, antibody-based screening, protein-binding assays and protein biochips. Protein biochips were described earlier in this chapter. 2D PAGE is combined with mass spectroscopy-based sequencing

techniques, which identifies both the amino acid sequences of proteins and their posttranslational appendages. This approach is combined with database search algorithms to sequence and characterize individual proteins.

### 2D Gel Electrophoresis

2D gel electrophoresis (2DGE) offers the highest resolution separations available for protein components of cells when gels of sufficient size are used. Proteins are separated in the first dimension on the basis of their charge and in the second dimension on the basis of their molecular mass. 2DGE is still the workhorse for obtaining protein expression patterns in cells. In high-format mode, it can produce gels containing up to 10,000 distinct proteins and peptide spots. The major problem with this technique is that most of the spots cannot be sequenced as they are beyond the capacity of current high-sensitivity sequencers. Standard format 2D gels yield up to 2,000 spots and are easy to sequence. During 2D PAGE (polyacrylamide gel electrophoresis), the proteins are separated in two dimensions (by isoelectric focusing and mass) and a pattern is achieved that places each of the 2,000 proteins of the cell at a grid reference point. By reference to the databases, individual proteins on the map can be identified as the product of genes that have been sequenced.

While comparing different samples, controlling the position of the protein spots can be critical and is completely dependent upon the fidelity of the isoelectric focusing first dimension and the molecular weight separating gel slab of the second dimension. Differences between the test samples are determined by quantifying the ratios of spot intensities in independent 2D gels and techniques such as mass spectrometry (MS) can then be used to help identify the proteins through peptide mass fingerprinting or direct sequencing. A number of variations in the basic 2DGE technology have enhanced separation of protein components in a sample.

Although 2DGE is the most widely used tool for separating proteins in expression proteomics, it is not without its limitations. Challenges faced when utilizing this technology are co-migration of proteins, systematic exclusion of highly hydrophobic molecules, and problems with detecting very acidic, very basic, very small, very large, or low abundance proteins. To meet the demands of protein separation, companies are developing new technologies that appear to be inexpensive and reliable, generate high-resolution protein separation and yield good visual detection of subtle differences. Competing emerging technologies such as capillary electrophoresis, capillary electrochromatography and ultra-HPLC (high performance liquid chromatography) are beginning to replace 2DGE.

### Mass Spectrometry

Mass spectrometry (MS) is the measurement of molecular mass. A mass spectrometer consists of three essential parts: (1) an ionization source with conversion of molecules into gas-phase ions; (2) a mass analyzer to separate individual mass to charge rations (m/z); and (3) an ion detector. Several variants of MS are described in the following sections (*see* Fig. 2.7).

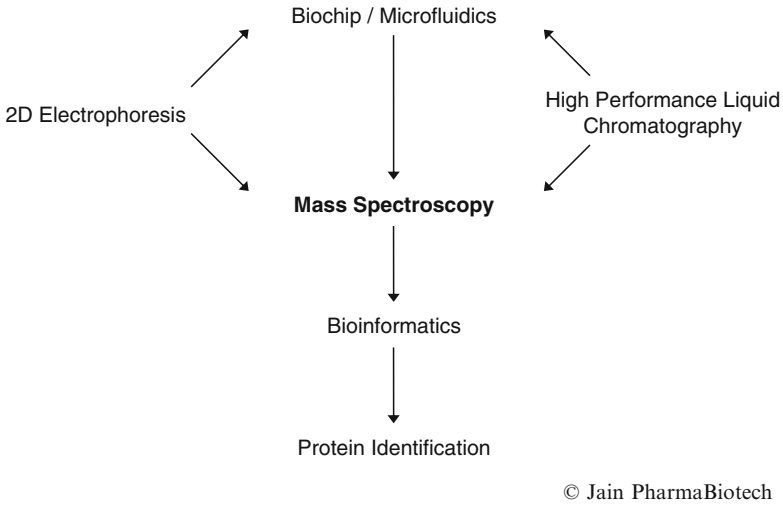


**Fig. 2.7** The central role of spectrometry in proteomics

### 2D PAGE and Mass Spectrometry

The essence of 2D PAGE (poly-acrylamide gel electrophoresis) approach is to separate proteins from a specific cell or tissue type, record the pattern, and then produce a Western Blot. Proteins in the blot are digested with a proteolytic enzyme, which has well-defined cleavage specificity. Peptide fragments can be analyzed by Matrix-Assisted Laser Desorption Mass Spectrometry (MALDI-MS). The resulting peptide masses are then compared with theoretical masses calculated from amino-acid sequence databases. This technique has been used successfully to identify yeast proteins. For completely sequenced genomes, 90 % of the proteins can be identified rapidly and automatically by searching databases with lists of peptide masses obtained by 2D gel technique and matrix-assisted laser description ionization. This study established that mass spectrometry provides the required throughput, the certainty of identification, and the general applicability to serve as the method of choice to connect genome and proteome. Nanoelectrospray tandem MS is then used to characterize novel proteins either by searching EST databases with peptide sequence tags, or by generating sufficient sequence for cloning. This approach can be automated.

## *Comparison of Proteomic and Genomic Approaches in Personalized Medicine*

Although proteomic and genomic approaches can be complementary, there are some similarities and differences that are shown in Table 2.4.

**Table 2.4** Comparison of proteomic and genomic approaches in personalized medicine

| Genotype/haplotype | Gene/protein expression | Protein function studies | Metabonomics |
|---|---|---|---|
| Polymorphisms related to a specific level of enzyme activity | Protein function is inferred from expression levels of mRNA or protein | Direct measurement of protein function | Infers level of protein function from metabolic profile |
| Genotype does not always correlate with protein function | Gene/protein expression does not always correlate with protein expression/ protein function | Direct measurement of protein function under conditions which mimic drug exposure | Levels of endogenous metabolites rather than exogenous levels; under static conditions |
| Does not account for polypharmacy, inducers and inhibitors | Does not account for polypharmacy, inducers and inhibitors | Accounts for polypharmacy, inducers and inhibitors | Accounts for polypharmacy, inducers and inhibitors |
| Qualitative | Quantitative | Quantitative | Qualitative |
| Identifies polymorphism found to correlate to fast or slow phenotype | Identifies increased or decreased expression of mRNA or protein | Identifies responders, non- responders, and those that will experience toxicity at standard doses | Identifies responders, non- responders, or those that will experience toxicity |
| Allows semi categorical individualization | Lack of correlation, makes individualization inaccurate | Allows accurate individualization of therapy to treat many of those originally identified as non-responders or at risk for toxicity | Non-responders or those that will experience toxicity are not treated with specific agent |



## Gene Expression Profiling

The activity of a gene, so called gene "expression" means that its DNA is used as a blueprint to produce a specific protein. The first step of gene expression is transcription, the process by which the sequence of DNA bases within a gene is used as a template to synthesize mRNA. Following transcription, the nascent mRNA is processed and transported out of the nucleus and into the cytoplasm of the cell. Once in the cytoplasm, the mature mRNA is engaged in the last step in gene expression, translation – the process by which proteins are synthesized. Finally there is post-translational modification of proteins into mature forms. Each of these steps in gene expression is subject to precise cellular controls that collectively allow the cell to respond to changing needs.

**Table 2.5** Selected methods for gene expression profiling

| |
|---|
| **Genome-wide methods** |
| Microarrays: whole genome expression array |
| Serial analysis of gene expression (SAGE) |
| Expressed sequence tags (ESTs) analysis |
| Gene expression profiling based on alternative RNA splicing |
| Tangerine expression profiling |
| **Individual sequences** |
| Real time RT-PCR |
| Competitive RT-PCR |
| RNase protection assay |
| T cell receptor expression analysis |
| **Analysis of single-cell gene expression** |
| Techniques for increasing cDNA yield from single-cell reverse transcription reactions |
| **RNA amplification** |
| **Monitoring in vivo gene expression** |
| Magnetic resonance imaging (MRI) |
| **Microarray/biochip/microfluidic technologies** |
| MAUI (MicroArray User Interface) hybridization |
| **QDot nanobarcodes** |



Less than half of all genes are expressed in a typical human cell, but the expressed genes vary from one cell to another and from one individual to another. Gene expression is used for studying gene function. Gene expression profiling, therefore, is relevant to personalized medicine. The temporal, developmental, typographical, histological and physiological patterns in which a gene is expressed provide clues to its biological role. All functions of cells, tissues and organs are controlled by differential gene expression. Malfunctioning of genes is involved in most diseases, not only inherited ones. Knowledge of which genes are expressed in healthy and diseased tissues would allow us to identify both the protein required for normal function and the abnormalities causing disease. This information will help in the development of new diagnostic tests for various illnesses as well as new drugs to alter the activity of the affected genes or proteins. Gene expression profiling is relevant to development of personalized medicine and some of the technologies used will be described briefly. Various techniques for detection of gene expression are shown in Table 2.5.

## *DNA Microarrays*

DNA microarrays have become the main technological workhorse for gene expression studies. The technology has advanced to such a point that researchers now demand microarrays that are cost-effective and have flexibility and quality assurance.

Although there are other, non-array methods for analyzing gene expression, such as serial analysis of gene expression (SAGE), the simplicity of the oligonucleotide approach makes it the most attractive option for the gene expression profiling. Important applications are in drug discovery, a field that is now flooded with potential targets. Microarrays will play an essential role in overcoming this obstacle in both target identification and in the long road of drug discovery and development. Two important therapeutic areas for gene expression profiling using microarrays are cancer and neurological disorders.

## *Analysis of Single-Cell Gene Expression*

Analysis of single-cell gene expression promises a more precise understanding of human disease pathogenesis and has important diagnostic applications. Single cell isolation methods include flow cytometry cell sorting and laser capture microdissection. Besides the gene expression analysis, the following nucleic acid amplification methods are suitable for single-cell analysis:

- Single cell phenotyping
- Homomeric tailed PCR, which allows unbiased amplification of RNA
- RNA amplification

Gene expression analysis of single cells is providing new insights into disease pathogenesis, and has applications in clinical diagnosis. Molecular signatures of some diseases can best be discerned by analysis of cell subpopulations. Studies in disease-relevant cell populations that identify important mRNA (and protein) differences between health and disease should allow earlier diagnosis, better therapeutic intervention and more sensitive monitoring of treatment efficacy. This will facilitate the developed of personalized medicine based on the molecular signatures of the diseased cell population.

Current assays for gene expression destroy the structural context. By combining advances in computational fluorescence microscopy with multiplex probe design, expression of many genes can be visualized simultaneously inside single cells with high spatial and temporal resolution.

## *Gene Expression Profiling Based on Alternative RNA Splicing*

RNA splicing is an essential, precisely regulated process that occurs after gene transcription and before mRNA translation. A gene is first transcribed into a pre-mRNA, which is a copy of the genomic DNA containing intronic regions destined to be removed during pre-mRNA processing (RNA splicing), as well as exonic sequences that are retained within the mature mRNA. During splicing, exons can either be retained in the mature message or targeted for removal in different combinations to

create a diverse array of mRNAs from a single pre-mRNA, a process referred to as alternative RNA splicing. Splicing is the crucial and tightly regulated step between gene transcription and protein translation. Alternative splicing could be responsible for generating up to three times as many proteins as the ~19,000 genes encoded by the human genome. The ability to analyze RNA splicing events gives a unique understanding of the sequences that are critical for normal cellular function. The control of alternative RNA splicing can be deregulated in human disease as a consequence of alterations within signaling cascades, within the spliceosome machinery or within the genes that are spliced.

Alterations in RNA splicing have a significant impact on drug action and can be exploited to generate pharmacogenomics tools in several ways.

- Alteration of alternative RNA splicing events triggered by drug or chemicals action constitutes a route through which relevant candidate genes can be selected for further genotyping because these genes are likely to lie within crucial pathways of drug action.
- Analyses of RNA splicing might provide a rapid method for detection of polymorphisms across the whole gene.
- RNA splicing alteration libraries between responders and non-responders would constitute a discovery tool for pharmacogenomically relevant SNPs.

## *Gene Expression Analysis on Biopsy Samples*

Patient outcomes are frequently known for people whose biopsy samples have been archived and gene expression analysis of these samples could provide a wealth of additional information. Analyzing these samples could help scientists determine why patients did or did not respond to the treatments they were given and provide greater understanding of which genes are involved in disease mechanisms. Fortunately, hospitals have collected millions of clinical tissue samples over the past few decades because they are required to store tumor samples from surgical procedures in case need arises for further testing. However, the standard procedure for preserving these samples involves immersing the tissue in formalin and embedding it in paraffin wax. Formalin-fixing, paraffin-embedding (FFPE) preservation process destroys, modifies, or degrades the nucleic acids, specifically the DNA and RNA, in biopsy samples. However reagents are available for extraction of nucleic acids from FFPE samples to enable gene expression studies.

## *Profiling Gene Expression Patterns of White Blood Cells*

White blood cells (WBCs) express tens of thousands of genes, whose expression levels are modified by genetic and external factors. Blood genomic profiles, created from distinct gene expression patterns of WBCs obtained by microarray

examination of a minimally invasive blood sample, can provide biomarkers of several different disease states. These profiles may be used for diagnostic, prognostic and therapeutic evaluations and also provide a method for the evaluation of the safety and efficacy of various therapeutics. Gene expression fingerprints are useful tools for monitoring exercise and training loads and thereby help to avoid training-associated health risks.

There is marked alteration in WBC gene expression in animal models of injury and inflammation; the majority of the differentially expressed genes appear to be uniquely associated with the type of injury and/or the inflammatory stimulus. Although some pathological states such as hypoxia may have direct impact on white blood cells that is manifested by specific expression profiles, seemingly unrelated events affecting various organs can markedly alter white blood cell gene expression in a predictable, characteristic way that provides a novel approach to diagnosis of diseases such as those involving the nervous system. Distinct human white blood cell genomic profiles have been reported for the following neurological disorders:

- Neurofibromatosis type 1, an autosomal dominant genetic disease caused by mutations of the NF1 gene at chromosome 17q11.2.
- Tourette's syndrome
- Migraine
- Epilepsy patients being treated with valproic acid
- Stroke

## *Serial Analysis of Gene Expression*

Serial analysis of gene expression (SAGE) technology was developed at the Johns Hopkins University (Baltimore, MD) and licensed to Genzyme. Three principles underlie the SAGE technology:

1. One short oligonucleotide sequence from a defined location within a transcript ("tag") allows accurate quantitation
2. Tag size (10–14 bp) is optimal for high throughput while maintaining accurate gene identification and quantitation.
3. The combined power of serial and parallel processing increases data throughput by orders of magnitude when compared to conventional expressed sequence analysis.

Important uses of this test include the study of differences in gene expression between cancer cells and their normal counterparts and identification of genes that may serve as useful diagnostic and prognostic markers. Differences in gene expression seen in SAGE translate directly into RNA differences as assessed by Northern blot analysis, and alterations identified in a few samples are consistent with data from a larger sample of primary tumor isolates. Gene expression monitoring by SAGE reduces the set of genes that are candidates for functional studies from tens

of thousands that are expressed in cancers to a few hundred or less that show significant disparity under comparative conditions. Analysis of gene expression differences in treatment responders versus non-responders could delineate differences between various patient populations and provide insight into the mechanism of action of different treatments. Gene expression patterns can also be useful in identifying new targets for therapeutic agents. SAGE helps to identify molecular differences, which correlate with adverse or beneficial response to drugs. Public sources of SAGE data, in particular through the Cancer Genome Anatomy Project, increase the value of this technology by making a large source of information on many tumors and normal tissues available for comparison.

### *Monitoring In Vivo Gene Expression by Molecular Imaging*

Molecular imaging is an emerging field of study that deals with imaging of disease on a cellular and molecular level. It can be considered as an extension of molecular diagnostics. Technologies encompassed within molecular imaging include optical, magnetic resonance imaging (MRI) and nuclear medicine techniques. In contradistinction to "classical" diagnostic imaging, it sets forth to probe the molecular abnormalities that are the basis of disease rather than to image the end effects of these molecular alterations. Radionuclide imaging, MRI, and positron emission tomography (PET) can be used visualize gene expression. Work done at the Beckman Institute/California Institute of Technology (Pasadena, CA) deals with 3D MRI image of gene expression based on intracellular messenger concentration.

Several current in vitro assays for protein and gene expression have been translated into the radiologic sciences. Endeavors are under way to image targets ranging from DNA to entire phenotypes in vivo. The merging fields of molecular biology, molecular medicine, and imaging modalities may provide the means to screen active drugs in vivo, image molecular processes, and diagnose disease at a presymptomatic stage.

## Molecular Imaging and Personalized Medicine

PET is the most sensitive and specific technique for imaging molecular pathways in vivo in humans. PET uses positron emitting radionuclides to label molecules, which can then be imaged in vivo. The inherent sensitivity and specificity of PET is the major strength of this technique. Indeed, PET can image molecular interactions and pathways, providing quantitative kinetic information down to sub-picomolar levels. Generally, the isotopes used are short-lived. Once the molecule is labeled, it is injected into the patient. The positrons that are emitted from the isotopes then interact locally with negatively charged electrons and emit what is called annihilating radiation. This radiation is detected by an external ring of detectors. It is the

timing and position of the detection that indicates the position of the molecule in time and space. Images can then be constructed tomographically, and regional time activities can be derived. The kinetic data produced provide information about the biological activity of the molecule. Molecular imaging provides in vivo information in contrast to the in vitro diagnostics. Moreover, it provides a direct method for the study of the effect of a drug in the human body. Molecular imaging plays a key role in the discovery and treatment process for neurological diseases such as Alzheimer's and cancer. The ability to image biological and pathological processes at a molecular level using PET imaging offers an unparalleled opportunity to radically reform the manner in which a disease is diagnosed and managed. Its translation into clinical practice will impact upon personalized medicine.

## Combination of Diagnostics and Therapeutics

The term "theranostic" is used to denote linking of a diagnostic to therapeutic. The second half of the word "nostic" is supposed to represent diagnostic but sounds more like "gnostic", a word introduced into English from Latin and meaning "having knowledge of". Theranostic could thus mean having knowledge of therapy. Moreover "theranostic" is confusing and not understood by most people. There is no difficulty in describing this concept without using a special term. Diagnostics used to guide therapeutics are also called "companion diagnostics". If one has to use a single word to describe a test linked to therapy, one can use "pharmacodiagnostic", which is more appropriate and easier to understand. This textbook does not recommend the use of the term "theranostic".

### *Use of Molecular Diagnostics for Stratification in Clinical Trials*

One of the problems in clinical trials is that response to a drug may vary considerably among patients. Some patients are poor or moderate responders, or the drugs themselves work well only in subsets of those treated. Efficacy and safety decisions are based on overall results of randomized clinical trials in which the therapeutic outcomes and clinical utility of a drug are averaged for entire populations. It is not possible to predict usefulness of such drugs in individual patients.

Stratification is a method of classifying patients based on clinically relevant genetic and/or immunological information during clinical trials of therapies and later application in practice. Such stratification is performed by the evaluation of clinically meaningful information generated by an appropriate molecular diagnostic test, which can guide in differentiating responders from non-responders to a therapy and selecting patients for treatment. This may be useful for matching the right drug to the right patient.

## *Companion Diagnostics*

Regardless of the name, the combination of diagnosis with therapy will have a major impact on the diagnostics industry, and the companies that are linking their diagnostic know-how with therapy are creating a new market place. The first companion diagnostic, Hercep-Test (DAKO), an immunohistochemistry assay used to identify patients with HER2-positive metastatic breast cancer, was launched in 1998 when it was discovered that patients with HER2 amplification responded better to Genentech's breast cancer therapy, Herceptin. The field of companion diagnostics is growing as more and more biomarkers are being discovered and validated. As of 2014, FDA has recommended companion diagnostics for ~100 drugs and requires biomarker/companion diagnostic information in the label of 23 approved drugs (17 for oncology) listed in Table 2.6.

**Table 2.6** Drugs requiring biomarker/companion diagnostic information in the label

| Drug | Therapeutic area | Biomarker(s) |
|---|---|---|
| Afatinib (Gilotrif) | Oncology: NSCLC | EGFR: deletion of exon 19 or substitution in exon 21 |
| Arsenic trioxide | Oncology | PML/RARα |
| Atorvastatin | Metabolic & endocrinology | LDL receptor |
| Brentuximab/Vedotin | Oncology | CD30 |
| Cetuximab (Erbitux) | Oncology | EGFR, KRAS |
| Crizotinib | Oncology | ELK |
| Dabrafenib (Tafinlar) | Oncology: melanoma | BRAF V600 |
| Dapsone | Dermatology & dental | G6PD |
| Dasatinib | Oncology | Philadelphia chromosome |
| Fulvestrant | Oncology | ER receptor |
| Imatinib (Gleevec) | Oncology | C-Kit, FIP1L1-PDGFRa fusion, Philadelphia chromosome, PDGFR gene rearrangement |
| Lapatinib | Oncology | Her2/neu |
| Maraviroc | Antiviral | CCR5 |
| Nilotinib | Oncology | Philadelphia chromosome |
| Panitumumab | Oncology | EGFR, KRAS |
| Sodium phenylacetate & sodium benzoate | Gastroenterology | NAGS; CPS; ASS, OTC, ASL; ARG |
| Sodium phenylbutyrate | Gastroenterology | NAGS; CPS; ASS, OTC, ASL; ARG |
| Tamoxifen | Oncology | ER |
| Tositumomab | Oncology | CD20 antigen |
| Trametinib (Mekinist) | Oncology: melanoma | BRAF V600 |
| Trastuzumab (Herceptin) | Oncology | Her2/neu |
| Vemurafenib (Zelboraf) | Oncology | BRAF V600 |

Updated in 2014 from FDA table of pharmacogenomic biomarkers in drug labels, 2012

An example of drug with recommendation for companion diagnosis in label information that lists genetic susceptibility relating to efficacy or dose is warfarin; labeling now includes FDA-recommended genotyping for mutations in two genes that cause increased susceptibility to bleeding, but the tests are not obligatory. Companion diagnostics for anticancer drugs are described in the chapter on cancer.

## Point-of-Care Diagnosis

Point of care (POC) or near patient testing involves analytical patient testing activities provided within the healthcare system, but performed outside the physical facilities of the clinical laboratories. POC does not require permanent dedicated space, but instead includes kits and instruments, which are either hand carried or transported to the vicinity of the patient for immediate testing at that site. Sites where POC may be performed include doctor's office, bed side in case of hospitalized patients, the emergency room or a disaster or war zone. POC may be performed in the field for several other indications including screening of populations for genetic disorders and cancer. The patients may even conduct the tests themselves at home. The most important application of molecular diagnostics is estimated to be at the POC and rapid results are required. POC diagnosis is important for the development of personalized medicine and various applications are listed in Table 2.7.

There are many reasons for the substantial growth of POC testing, but perhaps the most significant is that the accuracy and reliability of POC tests now approaches that of high-volume analyzers used in clinical laboratories. For physicians, the benefit of being able to obtain test results quickly at the bedside or in a critical care setting often outweighs the somewhat higher cost per test associated with POC testing. This is particularly true in the coronary care units of hospital emergency departments, where new cardiac biomarker tests can provide rapid results that physicians can use to make critical patient management decisions. The demand for POC tests has also stimulated an increase in their diversity. A small variety of home tests such as ovulation predictors, pregnancy tests, fecal occult blood assays, and blood glucose monitors have been available for years. More recently, the FDA has approved home-use tests for monitoring bladder cancer, anticoagulation therapy, urinary tract infections, HIV status, drugs of abuse, and even risk assessment for preterm labor and delivery.

POC diagnosis is well known with simple biochemical tests such as blood glucose monitoring. Hand-held diagnostic devices, biochips and electrochemical devices for the detection of DNA are particularly suited for POC diagnostics. Protein biochips, particularly microfluidic immunoassays, appear to be likely to get to POC first as several technical problems associated with use of nucleic acid biochips outside the laboratory are being worked out. Biochip and microfluidic technologies are also used for miniaturizing other laboratory tests such as cell count and automated immunoassays. Continued improvements in biosensor technology and miniaturization will increase the ability to test for many analytes at POC. Nanobiotechnology

**Table 2.7** Applications of point-of-care diagnosis

| |
|---|
| **In the hospital** |
| Emergency room testing for various pathogens in 'untested' blood donations |
| Rapid tests in emergency departments for microorganisms in severe diarrhea, meningitis etc. |
| Intensive care |
| Operating room |
| **In the physician's office** |
| Testing for viruses causing coughs and colds |
| Detection of bacterial infections to select appropriate antibiotic |
| Screening for cancer |
| **In field studies** |
| Screening of populations for genetic disorders |
| Testing of patients in clinical trials |
| Detection of microorganisms that are associated with bioterrorism |
| Identification of patients with communicable diseases at the point of immigration |
| Food testing |
| **In the home** |
| Self testing by the patient |
| Testing at home by visiting healthcare personnel |



will facilitate development of POC tests. Nanotechnology-based diagnostics provides the means to monitor drugs administered by nanoparticle carriers. Nano-diagnostic biosensors might be incorporated in nanorobotic devices for navigating the body to detect and destroy viruses or cancer cells (Jain 2012).

## *Advantages Versus Disadvantages of Point-of-Care Diagnosis*

Advantages of POC diagnosis are:

- Appropriate immediate prescribing according to diagnosis
- Rapid implementation of measures for control of infections
- Decreased dependency of remote areas on distant diagnostic facilities
- Rapid diagnosis, alleviating unnecessary anxiety associated with waiting for results
- Contributing to decreased overall cost of health care by reducing inappropriate treatments while waiting for traditional laboratory diagnosis
- No need for transport of specimens

Disadvantages of POC diagnosis are:

- Misuse or misinterpretation of test result, particularly if used in the home
- Overutilization of services leading to rise of cost of health care
- Potential loss of epidemiological data
- Less opportunity for large scale automation

- Inadequate discussion or patient counseling
- Reduced opportunity for internal and external quality assurance, with associated risk of misdiagnosis
- Medicolegal implications

### *Future Prospects of Point-of-Care Diagnosis*

POC-testing is destined to become a major force in the development of healthcare delivery. Currently, POC devices account for approximately a quarter of the worldwide market for clinical laboratory IVD products. Advances will be on four fronts:

1. Scope: expanding the POC format into new categories of in vitro diagnostic testing
2. Connectivity: communicating test results externally with ease and flexibility
3. Non-invasiveness: improving the way test samples are obtained from the body.
4. Miniaturization: reducing the size of the devices to enable novel uses

The major technological requirements to reduce complications of POC have been identified by both the manufacturers and the regulators. These focus on reduction of dependence on the operator and seamless automation of quality control.

## Genetic Testing for Disease Predisposition

Genetic testing is a broad term, which covers several techniques, including those used to determine paternity and in forensic medicine. However, most genetic tests are used to confirm a suspected diagnosis, to predict susceptibility to an illness, to identify individuals who carry a specific genetic mutation but remain unaffected themselves, or to predict how an individual is likely to respond to a certain therapy. Genetic tests are also used to screen fetuses, newborns, and embryos used in in vitro fertilization for genetic defects. Over 1,800 genetic tests are available including those that indicate susceptibility to cancer, neurological disorders, and heart disease.

An understanding of the disease-related effects of specific genetic variants provides the basis for direct genetic testing in individuals and alleviates reliance on population categories to improve disease diagnosis and treatment (Rotimi and Jorde 2010). Testing for gene mutations that confer susceptibility to adult-onset disorders has potential benefits, but these must be balanced against the psychological harms, if any. The published findings on the psychological effects of such testing, focusing on Huntington's disease, which has the most available data, and the hereditary cancer syndromes. Most of the evidence suggests that non-carriers and carriers differ significantly in terms of short-term, but not long-term, psychological adjustment to test results. The psychological impact of genetic testing depends more on pretest psychological distress than the test result itself.

### *Preventive Genetics by Early Diagnosis of Mitochondrial Diseases*

Diseases resulting from mutations in mitochondrial DNA are common in both adults and children. Most mitochondrial disease may go undiagnosed because a primary care physician does not suspect the disease or because the causative mutation is missed by current routine diagnostic methods. MitoDx™ (MEDomics Inc) is an innovative test for early diagnosis of mitochondrial diseases using next generation sequencing technology (SOLiD from Life Technologies) to detect all mutations in any of the 37 mitochondrial DNA genes. Because cells contain hundreds of mtDNA molecules, any particular tissue may contain mtDNA molecules that are all identical, or there may be a fraction that differs. When both normal and mutant molecules exist, the mitochondria are said to be heteroplasmic. The heteroplasmic fraction of mutations can differ substantially among tissues. Low levels of heteroplasmy in blood are generally not detected by standard methods, but are detected by the MitoDx™ test even at levels as low as 1 %. Diagnosis of mitochondrial disease can enable life-saving therapy decisions and accurate family risk counseling. This is guided by experts and is different from personal genetic service offered by various companies shown in the following section.

### *Direct-to-Consumer Genetic Services*

A large number of companies offers test to screen for diseases with a genetic component or to identify those at risk of developing a certain disease. Some of the companies developing genetic tests are mentioned in other categories such as those involved in prenatal and cancer diagnostics. Commercialization of genetic technologies is expanding the horizons for the marketing and sales of direct-to-consumer (DTC) genetic tests. Several companies are involved in this activity. Some companies have made available DTC “personal genome services” that rely on the same arrays of 500,000 to 1 million SNPs used in genome wide association studies. Essentially, a client sends a DNA sample to one of these companies, which analyzes the sample by means of SNP array; the data are stored in an online private account, the results are compared with allele-phenotype databases maintained and updated by the company, and the customer receives readout of his or her levels of risk for specific conditions.

## Future of Molecular Diagnostics in Personalized Medicine

Most cells are healthy, but they can become cancerous, get infected by viruses, and undergo cycles as well as aging. Single cell analysis will be important for development of personalized treatments that target disease at the cellular level. Trend in

current research awards for future projects are to validate and refine established technologies including those to detect genetic changes in live animals, detect the slightest differences in genetic variation, and profile gene expression in a cell's nucleus to identify early protein signatures. Examples are gene expression sensors that detect environmentally triggered changes among cells in living tissue and technologies that uncover how a gene regulator exerts effects on different classes of target genes.

A wide variety of drugs in late preclinical and early clinical development are being targeted to disease-specific gene and protein defects that will require co-approval of diagnostic and therapeutic products by regulatory agencies. An increasingly educated public will demand more information about their predisposition for serious diseases and how these potential illnesses can be detected in an early stage when they can be arrested or cured with new therapies custom-designed for their individual clinical status. To respond to this demand, major pharmaceutical companies will partner with diagnostics companies or develop their own in-house capabilities that will permit efficient production of more effective and less toxic integrated personalized medicine drug and test products. For clinical laboratories and pathologists, this integration of diagnostics and therapeutics represents a major new opportunity to emerge as leaders of the new medicine, guiding the selection, dosage, route of administration, and multidrug combinations and producing increased efficacy and reduced toxicity of pharmaceutical products.

Advances in new technologies such as nanobiotechnology have not only refined molecular diagnosis but facilitated its integration with targeted drug delivery for development for personalized medicine. Role of nanobiotechnology is described in Chap. 7.

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# Chapter 3
# Role of Biomarkers in Personalized Medicine

## Introduction

A biological marker (biomarker) is simply a molecule that indicates an alteration in physiology from normal. For example, any specific molecular alteration of a cancer cell either on DNA, RNA, or protein level can be referred to as a molecular biomarker. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. The topic of biomarkers has been discussed in a book as well as a special report on this topic (Jain 2010, 2015). Biomarkers are further described in several chapter of this book. The expression of a distinct gene can enable its identification in a tissue with none of the surrounding cells expressing the specific biomarker. Impact of biomarkers on personalized medicine is shown schematically in Fig. 3.1.

## Biomarkers and Diagnostics

Currently available molecular diagnostic technologies have been used to detect biomarkers of various diseases such as cancer, metabolic disorders, infections and diseases of the central nervous system. Some of the newly discovered biomarkers also form the basis of innovative molecular diagnostic tests. Those relevant to personalized medicine may be categorized as pharmacogenetic tests or pharmacogenomic tests.

A pharmacogenetic test is an assay intended to study interindividual variations in DNA sequence related to drug absorption and disposition (pharmacokinetics) or drug action (pharmacodynamics), including polymorphic variation in the genes that encode the functions of transporters, metabolizing enzymes, receptors, and other proteins.

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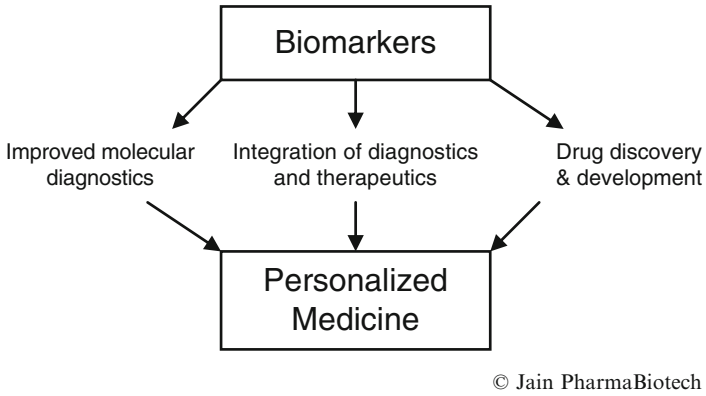


**Fig. 3.1** Impact of biomarkers on personalized medicine

A pharmacogenomic test is an assay intended to study interindividual variations in whole-genome or candidate gene, SNPs, haplotype markers, or alterations in gene expression or inactivation that may be correlated with pharmacological function and therapeutic response. In some cases, the pattern or profile of change is the relevant biomarker, rather than changes in individual markers.

Diagnostic systems such as DNA microarrays and proteomics enable simultaneous assessment of multiple biomarkers. Progress made in recent years suggests that pharmacogenomic biomarkers have the potential to provide physicians with clinically useful information that can improve patient care through increased individualization of treatment, particularly in the management of life-threatening disease.

## Expression Signatures as Diagnostic/Prognostic Tools

Gene expression signatures as determined by microarrays can be used as biomarkers for diagnosis as well monitoring of therapy. The best examples are in cancer. Gene expression signatures are used to refine molecular classification of breast cancer. Utilization of these signatures together with standard clinical parameters provides a unique combination to identify patients that respond to standard anthracycline chemotherapy, which has been validated. The proprietary eXpress Profiling™ multiplexed PCR technology (Althea Technologies Inc), which enables high throughput gene expression analysis, is being combined with bioinformatics to discover and apply gene expression signatures for a targeted disease or drug activity. This combination will provide advanced methods of data mining to extract biomarkers from the large gene expression data sets.

## Role of Biomarkers in Development of Personalized Drugs

In addition to personalizing the use of existing drugs, the development of new personalized drugs should start at the discovery stage. One example of this is pharmacogenetics/pharmacogenomics-based monoclonal antibody (MAb) drug development in oncology. Another example of the usefulness of biomarkers in development of personalized medicine is biomarkers for Huntington's disease (HD). Genome-wide gene expression profiles from blood samples of HD patients have identified changes in blood mRNAs that clearly distinguish HD patients from controls. The elevated mRNAs is significantly reduced in HD patients involved in a dose-finding study of the histone deacetylase inhibitor sodium phenylbutyrate. These alterations in mRNA expression correlate with disease progression and response to experimental treatment. Such biomarkers may provide clues to the state of HD and may be of predictive value in clinical trials.

The advantage of applying biomarkers to early drug development is that they might aid in preclinical and early clinical decisions such as dose ranging, definition of treatment regimen, or even a preview of efficacy. Later in the clinic trials, biomarkers could be used to facilitate patient stratification, selection and the description of surrogate endpoints. Information derived from biomarkers should result in a better understanding of preclinical and clinical data, which ultimately benefits patients and drug developers. If the promise of biomarkers is realized, they will become a routine component of drug development and companions to newly discovered therapies.

## Drug Rescue by Biomarker-Based Personalized Medicine

Biomarkers can rescue drugs by identifying the patients that respond to them. Herceptin, approved in 1998, emerged as a $480 million-per-year winner only a decade after clinical trials showed little or no efficacy. Only when the 20–30 % of women with breast cancer whose tumors overexpress HER2 were singled out, was the drug's efficacy indisputable. In the pivotal clinical trial of patients with metastatic breast cancer, tumor-response rates to Herceptin plus chemotherapy were 45 %, compared to 29 % for chemotherapy alone.

But response is not wholly predictable. Reported response rates for HER2-positive cancers vary from <20 % to >75 %. HER2-positive cells that do not respond to Herceptin may have more active forms of the kinase Akt, whereas HER2 belongs to a receptor family that can be activated by 11 different soluble proteins and combinations thereof. Investigation of the biology behind the biomarker is likely to improve treatment of breast cancer. Similarly, the lung-cancer drug Iressa (gefitinib) could be rescued by a diagnostic based on a biomarker. Unfavorable clinical trial results were disappointing, but finding the patients most likely to benefit improved the outlook. Various studies found that patients that responded to Iressa had mutations in the gene for EGFR.

## Biomarkers for Monitoring Response to Therapy

One of the important aspects of personalized medicine is the ability to monitor response to therapy. There are some examples in various diseases mentioned in chapters dealing with various diseases. A few examples are given here to show the value of biomarkers as well as their limitations in monitoring response to therapy.

Biomarkers are important tools for assessing the malignant potential of tumor cells and for establishing risk-stratified therapies. Proteomics biomarkers can predict in various types of cancer. For example, proteomic biomarker candidate, pfetin, is a novel prognostic biomarker in gastrointestinal stromal tumor, where the anticancer drug is available for reducing the risk of postoperative metastases. The prognostic utility of pfetin was immunohistochemically established by several validation studies, and it is expected that in the near future it will be possible to select patients who may need adjuvant therapy by measuring the expression of pfetin in surgical specimens (Kondo 2012).

Sensitive noninvasive strategies for monitoring treatment response in rheumatoid arthritis (RA) would be valuable for facilitating appropriate therapy and dosing, evaluating clinical outcome, and developing more effective drugs. Because different proteases are highly up-regulated in RA and contribute significantly to joint destruction, the suitability of such enzymes as in vivo imaging biomarkers for early evaluation of treatment response was investigated in a murine model of RA (Wunder et al. 2004). Using a protease-activated near-infrared fluorescence (NIRF) imaging "smart" probe, the presence and distribution of fluorescence in arthritic joints of mice with collagen-induced arthritis was examined by both noninvasive fluorescence imaging and histology. Proteases that target the Lys-Lys cleavage site, including cathepsin B, activate probe fluorescence. Treatment monitoring data, obtained following methotrexate therapy, showed that protease-activated NIRF probes are sensitive means of imaging the presence of target enzymes in arthritic joints and can be used for early monitoring of treatment response to antirheumatic drugs such as methotrexate.

Assessment of hepatic damage associated with chronic hepatitis B (CHB) currently relies on measurement of serum transaminases and assessment of hepatic histology. Serum hepatic function tests combined with liver fibrosis biomarkers – type IV collagen (CIV), amino-terminal propeptide of type I procollagen (PINP), amino-terminal propeptide of type III procollagen (PIIINP) and carboxy-terminal telopeptide of type I collagen (ICTP) – can be used for monitoring the effect of lamivudine therapy for CHB because PINP/ITCP ratio is sensitive and specific in detecting responders to treatment.

Serial measurements of biomarkers might be beneficial for assessing the adequacy of drug therapy in patients with advanced heart failure. Therapy guided by NT-proBNP, a biomarker of heart failure, might be helpful because it should be lowered by therapies that decrease endogenous BNP secretion. However, patients may not demonstrate biochemically significant decreases in NT-proBNP and BNP despite a clinical response to intravenous nesiritide. Until we know more about the responses of natriuretic peptides to therapies such as nesiritide, a strategy of monitoring NT-proBNP and BNP to guide therapy cannot be universally advocated.

## Bioinformatics to Sort Biomarker Data for Personalized Medicine

Bioinformatics methods are being applied for the development and validation of new genomic biomarkers that are useful for selecting the right treatments for the right patients. The established heterogeneity of disease based on genomic biomarkers requires development of new paradigms of design and analysis of clinical trials for assessing the validity and clinical utility of new treatments and the companion biomarkers in personalized medicine. Stratification prior to clinical trial would involve measurement of a relevant biomarkers and separation of the study population into biomarkers positive and biomarker negative groups; each group is randomized into those to be treated with a new drug vs control drug or placebo (Matsui 2013).

In 2012, the Center of Excellence for the Prevention of Organ Failure (PROOF) and IO Informatics started collaboration to develop a web-based software application addressing chronic heart, lung, and kidney diseases. The application will be developed so that clinicians can use it on handheld device and other technology, and it will be used with blood tests developed by the PROOF Center that target chronic disease and transplantation. The application will give an overall score indicating patient risk level and associated clinical recommendations to help guide decision making. The scores and recommendations will be based on gene expression data, protein expression data, and longitudinal clinical observations. Future applications of the technology will enable automated, pre-symptomatic screening for biomarker-based risk events, disease severity characterization, and treatments that are suitable for individual patients.

### *Use of Bayesian Approach in Biomarker-Based Clinical Trials*

Innovative clinical trial designs are needed to address the difficulties and issues in the development and validation of biomarker-based personalized therapies. A new clinical trial design that captures the strengths of the frequentist and Bayesian approaches has been proposed to address some of these issues (Lai et al. 2012). There are advantages of using likelihood inference and interim analysis to meet the challenges in the sample size needed and in the constantly evolving biomarker landscape and genomic and proteomic technologies.

The statistical method used nearly exclusively to design and monitor clinical trials today, a method called frequentist or Neyman-Pearson (for the statisticians who advocated its use), is so narrowly focused and rigorous in its requirements that it limits innovation and learning. A solution is to adopt a system called the Bayesian method, a statistical approach more in line with how science works (Berry 2006). The main difference between the Bayesian approach and the frequentist approach to clinical trials has to do with how each method deals with uncertainty, an inescapable

component of any clinical trial. Unlike frequentist methods, Bayesian methods assign anything unknown a probability using information from previous experiments. In other words, Bayesian methods make use of the results of previous experiments, whereas frequentist approaches assume we have no prior results. This approach is being put to the test at M. D. Anderson Cancer Center (Houston, TX), where more than 100 cancer-related phase I and II clinical trials are being planned or carried out using the Bayesian approach. The Bayesian approach is better for doctors, patients who participate in clinical trials and for patients who are waiting for new treatments to become available. Physicians want to be able to design trials to look at multiple potential treatment combinations and use biomarkers to determine who is responding to what medication. They would like to treat that patient optimally depending on the patient's disease characteristics. If interim results indicate that patients with a certain genetic makeup respond better to a specific treatment, it is possible to recruit more of those patients to that arm of the study without compromising the overall conclusions. Use of the Bayesian approach may make it possible to reduce the number of patients required for a trial by as much as 30 %, thereby reducing the risk to patients and the cost and time required to develop therapeutic strategies.

Using a Bayesian approach, contrary to the standard approach, the trial design exploits the results as the trial is ongoing and adapts based on these interim results. In order to have the personalized medicine, it will be necessary to be more flexible in how we evaluate potential new treatments. Moreover, it is possible to reduce the exposure of patients in trials to ineffective therapy using the Bayesian approach. Whether the Bayesian approach will gain acceptance in clinical trials depends a lot on its acceptance by the FDA in determining safety and efficacy of new treatments. The FDA has already approved the Bristol-Myers Squibb drug Pravigard Pac for prevention of secondary cardiac events based on data evaluated using the Bayesian approach.

## Concluding Remarks

The important points of role of biomarkers in development of personalized medicine are:

- Biomarkers will enable early diagnosis of disease to facilitate optimization of therapy.
- Biomarkers will play an important role in combining diagnosis with therapeutics – an important feature of personalized medicine.
- There will be an increase in the number of new drugs suitable for personalized treatment, which will be discovered by use of biomarkers.
- Validated biomarkers will play an increasing role in clinical trials for personalizing therapeutics.
- Biomarker-based monitoring of drug efficacy will guide personalized management of several diseases.

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# Chapter 4
# Pharmacogenetics

## Basics of Pharmacogenetics

Pharmacogenetics, a term recognized in pharmacology in the pre-genomic era, is the study of influence of genetic factors on action of drugs as opposed to genetic causes of disease. Now it is the study of the linkage between the individual's genotype and the individual's ability to metabolize a foreign compound. The pharmacological effect of a drug depends on pharmacodynamics (interaction with the target or the site of action) and pharmacokinetics (absorption, distribution and metabolism). It also covers the influence of various factors on these processes. Drug metabolism is one of the major determinants of drug clearance and the factor that is most often responsible for interindividual differences in pharmacokinetics. Pharmacogenetics links genotype and phenotype as shown in Fig. 4.1.

The differences in response to medications are often greater among members of a population than they are within the same person or between monozygotic twins at different times. The existence of large population differences with small intrapatient variability is consistent with inheritance as a determinant of drug response. It is estimated that genetics can account for 20–95 % of variability in drug disposition and effects. Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications.

Although interindividual variations in drug response result from effects of age, sex, disease or drug interactions, genetic factors represent an important influence in drug response and efficacy and remain constant throughout life. This has led to the recognition of the discipline "pharmacogenetics" since the 1950s, which can be viewed an as integration of gene profiling and pharmaceutical chemistry. From this initial definition, the scope has broadened so that it overlaps with pharmacogenomics (see Chap. 5).

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## Role of Molecular Diagnostics in Pharmacogenetics

Molecular diagnostic technologies used for pharmacogenetics have been described in Chap. 2. Role of pharmacogenetic technologies in personalized medicine is shown in Fig. 4.2.

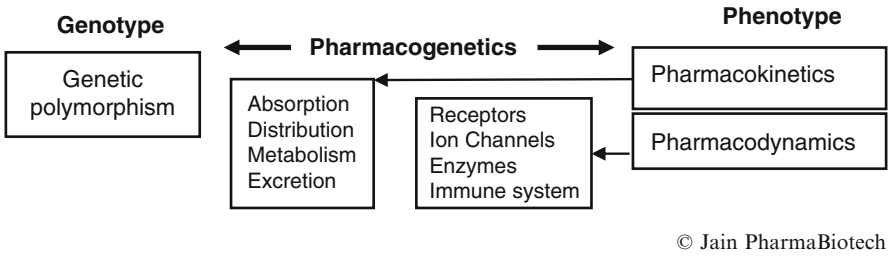


**Fig. 4.1** Pharmacogenetics as a link between genotype and phenotype

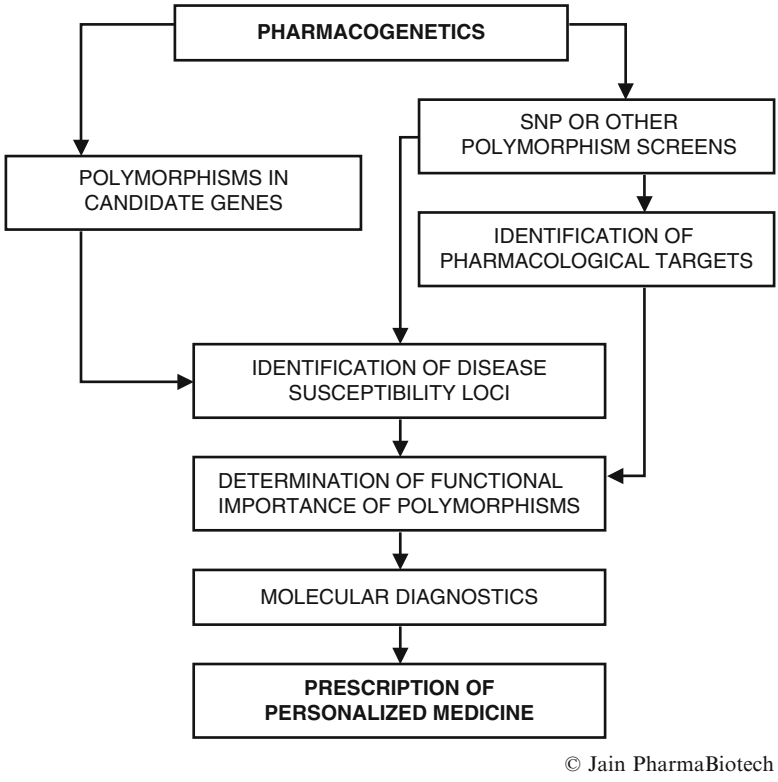


**Fig. 4.2** Role of pharmacogenetic technologies in personalized medicine

Genotyping involves identification of defined genetic mutations that give rise to the specific drug metabolism phenotype. These mutations include genetic alterations that lead to overexpression (gene duplication), absence of an active protein (null allele), or production of a mutant protein with diminished catalytic capacity (inactivating allele). Genetic mutations can be screened by molecular diagnostic methods. Although genome-wide association studies have long been considered an avenue for improving diagnostics, prognostics, and treatment, an important current application is in pharmacogenetics for improving drug safety and reducing adverse reactions to drugs.

## Role of Pharmacogenetics in Pharmaceutical Industry

Genes influence pharmacodynamics and pharmacokinetics. Sequence variations in drug disposition genes can alter the pharmacokinetics of a drug, while sequence variations in drug target genes can change the pharmacodynamics of the drug. The two most common strategies to test a pharmacogenetic question are the candidate-gene approach and genome wide association study. Pharmacogenetics has a three-fold role in the pharmaceutical industry, which is relevant to the development of personalized medicines:

1. For study of the drug metabolism and pharmacological effects.
2. For predicting genetically determined adverse reactions.
3. Drug discovery and development and as an aid to planning clinical trials.

### *Study of the Drug Metabolism and Pharmacological Effects*

Most drugs are metabolized to some extent. Metabolism results in detoxification or elimination of the drug or activation of the prodrug to the biologically active form. It may even result in the formation of toxic metabolites. From a pharmacological point of view, pathways of drug metabolism can be classified as either phase I reactions (oxidation, reduction and hydrolysis) or phase II conjugation reactions (acetylation, reduction and hydrolysis). Phase II reactions may occur prior to phase I and may not be followed by oxidation, reduction or hydrolysis.

### *Causes of Variations in Drug Metabolism*

Given the complex interplay among the many factors that influence a drug dose, determination of an appropriate dose of a particular drug for a given patient will eventually require knowledge about both genetic and nongenetic factors that affect

drug disposition and pharmacodynamics. Causes of variations in drug metabolism include the following:

- Individual factors such as age, sex, body fat and body weight
- Environmental factors such as pollutants, alcohol and smoking
- Physiological factors: e.g. function of liver, kidneys, lungs, and cardiovascular system.
- Genetic factors such as polymorphisms of drug metabolizing enzymes, drug transporters, drug receptors, ion channels and signal transduction pathways
- Concomitant drugs
- Concomitant diseases

Potential consequences of polymorphic drug metabolism are:

- Prolongation or intensification of pharmacological effect
- Adverse drug reactions
- Lack of prodrug activation
- Drug toxicity
- Lack of efficacy at prescribed dose requiring increase in dosage
- Metabolism by alternative, deleterious pathways
- Drug-drug interactions

It is of considerable importance to know the metabolic status of an individual, particularly when using drugs with a narrow therapeutic range. Differences in metabolism of drugs can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Inter- and intra-individual variability in pharmacokinetics of most drugs is largely determined by variable liver function as described by parameters of hepatic blood flow and metabolic capacity. Among the factors affecting these parameters are genetic differences in metabolizing enzymes. Glucose-6-phosphate dehydrogenase and N-Acetyltransferase were the earliest enzymes to be studied. Currently the most important of these are liver enzymes.

## *Enzymes Relevant to Drug Metabolism*

There are more than 30 families of drug-metabolizing enzymes in humans and essentially all have genetic variants, many of which translate into functional changes in the proteins encoded. For practical purposes these enzymes can be divided into phase I and phase II as shown in Table 4.1:

Overall, in poor metabolizers, whether phase I or phase II, there is limited metabolism in most patients unless another major metabolic pathway involving other enzymes exists. Drug metabolism also depends on whether the parent compound is a prodrug that forms an active metabolite, and poor metabolizers under this condition will form only trace amounts of an active compound.

**Table 4.1** Enzymes relevant to drug metabolism

| Phase I enzymes (predominantly oxidative) | Phase II enzymes (conjugative) |
|---|---|
| Alcohol dehydrogenase | N-acetyl transferase 2 |
| Cytochrome P (pigment)-450 (cyp) with subtypes | Catechol O-methyltransferase |
| Dyhydropyrimidine dehydrogenase | Glutathione-S-transferase and variants |
| Epoxide hydrolases | Sulfotransferases and variants |
| Flavine-dependent monooxygenase 3 | Thiopurine S-methyltransferase |
| NADPH-quinone oxidoreductase | Thiopurine S-methyltransferase |
| Pseudocholinesterase (butyrylcholinesterase) | Uridine diphosphate-glucuronosyltransferase 1A1 |



## *Pharmacogenetics of Phase I Metabolism*

The most important of these enzymes is the CYP450 group.

### CYP450

The cytochrome P450 enzyme system consists of a large family of proteins, which are involved, in the synthesis and/or degradation of a vast number of endogenous compounds such as steroids, cholesterol, vitamins and retinoic acid, as well as the metabolism of exogenous toxins. P450 enzymes can alter, abolish or enhance drug metabolism. There are likely more than 100 P450 genes that control these enzymes. The most frequent change observed in CYP2D6 is a polymorphism that results in an aberrant RNA splice event, which causes truncation and inactivation of the protein. AmpliChip CYP450 (Roche) enables clinical diagnostic laboratories to identify polymorphisms in two genes CYP2D6 and CYP2C19.

More than 50 % of the clinically used drugs are cleared through the action of P450 enzymes: CYP2D6 and CYP3A4 metabolize majority of these. Because cytochrome P450s play key roles in regulating important physiological processes, they are also attractive targets for drug discovery. Inhibitors of P450 enzymes are used clinically or are under evaluation for treatment of a number of diseases. Examples of genetic variations seen in three of the CYP450 enzymes and the clinical impact of those variations are shown in Table 4.2.

Clinically relevant genetic polymorphisms have been found in cytochrome P450-mediated oxidation of debrisoquine and sparteine (CYP2D6), which represents 25 % of the major isoforms of P450 responsible for drug metabolism. Frequency distribution of drugs metabolized by major CYP450 isoforms is shown in Table 4.3. Commonly prescribed medications, which are metabolized by CYP2D6, are shown in Table 4.4.

**Table 4.2** Examples of mutation of the enzyme CYP450

| CYP450 enzyme | Prototype substrate | Allele | Mutation |
|---|---|---|---|
| CYP2D6 | Debrisoquine | 2XN | Genetic duplication |
| | | 4 | Defective splicing |
| | | 10 | Gene deletion and single amino acid substitution |
| | | 17 | Single amino acid substitution |
| CYP2C19 | S-mephenytoin | 2 | Aberrant splice site |
| | | 3 | Premature stop codon |
| CYP2C9 | Phenytoin, tolbutamide, warfarin | 2 and 3 | Single amino acid substitution leading to altered substrate specificity |



**Table 4.3** Frequency distribution of drugs metabolized by major isoforms of CYP450

| Isoform of CYP450 | Frequency distribution of drugs metabolized (%) |
|---|---|
| CYP3A4 | 50 |
| CYP2D6 | 20 |
| CYP2C9 | 10 |
| CYP2C19 | 5 |
| CYP1A2, CYP2E1, CYP1A2 and unidentified forms | 15 |
| Total | 100 |



**Table 4.4** Commonly prescribed medications, which are metabolized by CYP2D6

| | | |
|---|---|---|
| Amiadarone | Fluvoxamine | Phenacetin |
| Amitriptyline | Haloperidol | Phenformin |
| Carvedilol | Imipramine | Propafenone |
| Chloropromazine | Indoramin | Propanolol |
| Clomipramine | Mefloquine | Quinidine |
| Clopidogrel | Methoxyphenamine | Risperidone |
| Clozapine | Metoprolol | Sertraline |
| Codeine | Nortriptyline | Tamadol |
| Desipramine | Olanzapine | Tamoxifen |
| Diltiazem | Paroxetine | Thioridazine |
| Encainide | Perazine | Timolol |
| Flencainide | Perhexilene | Tropisetron |
| Fluoxetine | Perphenazine | Venlaflaxine |

**CYP2C9** Two inherited SNPs termed CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) are known to affect catalytic function. About 35 % of the Caucasian population carries at least one *2 or *3 allele. CYP2C9 genotyping may be considered along with the use of nonsteroidal antiinflammatory drugs, oral hypoglycemics, vitamin K antagonistic oral anticoagulants, and phenytoin. However, before instituting the routine clinical use of genotyping, the benefits of genotype-based therapeutic recommendations need to be confirmed by randomized controlled clinical trials.

**CYP2C19** This is the gene encoding S-mephenytoin hydroxylase and its mutations lead to poor metabolism of the following drugs: amitriptyline, citalopram, clomipramine, diazepam, imipramine, mephenytoin, omeprazole, and propranolol.

**CYP3A** This subfamily comprises 3A3, 3A4, and 3A5 isoenzymes in the humans. Pharmaceutical substrates of this enzyme are: acetaminophen, alprazolam, carbamazepine, cyclosporine, diltiazem, erythromycin, lidocaine, lovastatin, nifedipine, tamoxifen, terfenadine, verapamil and vinblastine. Differences in the expression of the CYP3A family contribute to variability in the absorption and clearance of drugs as diverse as calcium channel blockers and HIV protease inhibitors.

Hepatic expression of CYP3A4 varies more than 50-fold among individuals. Polymorphisms in the CYP3A4 gene may explain the person-to-person variations seen in the intensity and duration of drug action as well as in the occurrence of side effects. Understanding the genetic basis of differences in CYP3A4 function will enable the determination of proper drug dosage for individual patients to achieve an optimal therapeutic response with minimal side effects.

Only individuals with the full-length CYP3A5 allele (CYP3A5*1) express large amounts of CYP3A5, whereas those with a truncated CYP3A5 express little or no CYP3A5. Because polymorphic CYP3A5 is one factor contributing to individual variation in CYP3A-mediated metabolism of drugs, simple DNA-based tests can now be used to determine how individual differences in CYP3A5 contribute to the overall metabolic fate of these CYP3A substrates, to their pharmacodynamic variability and to disease risk. Prospective patients would first be CYP3A5 genotyped, followed by targeted drug therapy i.e. tailoring the drug concentration to optimize systemic concentrations of drug and drug response. This is likely to be most relevant for drugs with narrow therapeutic indices primarily metabolized by CYP3As, including many anticancer and anti-transplant rejection drugs. This strategy will enable identification of those patients who are at risk associated with metabolizing the CYP3A5 substrate faster or slower so that the issue of CYP3A5-dependent variability in pharmacokinetics can be effectively addressed.

## P450 CYP 2D6 Inhibition by Selective Serotonin Reuptake Inhibitors

Most reports of metabolic enzyme inhibition by selective serotonin reuptake inhibitors (SSRIs) have focused on changes in concentration of the affected drug. For example, studies have addressed elevated desipramine concentrations with

paroxetine, increases in imipramine concentrations with fluvoxamine, and increased phenytoin concentrations with sertraline. Due to interindividual variability in drug disposition, plasma concentrations of SSRIs vary significantly among individuals. Change in enzyme activity as a result of drug-drug interaction may be equally clinically relevant for heterozygous extensive metabolizers (toward poor-metabolizer status) and homozygous extensive metabolizers (toward heterozygous extensive-metabolizer status). A possible cause of significant interindividual differences in the magnitude of CYP2D6 inhibition is the pharmacokinetic variability of the inhibitor itself. Another determinant of overall interaction magnitude is unbound drug concentration in plasma and hepatocytes. A similar extent of intersubject variability in hepatocyte drug concentration is likely at the site of enzyme inhibition.

There are positive and significant correlations between paroxetine and fluoxetine concentrations and CYP2D6 inhibition. These correlations illustrate the role of plasma concentrations and dosage on magnitude of enzyme inhibition. The potential of paroxetine, a CYP2D6 substrate, as an inhibitor may be further affected by specific genotype and basal metabolic capacity of individual subjects.

### Cytochrome P450 Polymorphisms and Response to Clopidogrel

Clopidogrel requires transformation into an active metabolite by cytochrome P450 (CYP) enzymes for its antiplatelet effect. A study has tested the association between functional genetic variants in CYP genes, plasma concentrations of active drug metabolite, and platelet inhibition in response to clopidogrel in healthy subjects (Mega et al. 2009). The investigators then examined the association between these genetic variants and cardiovascular outcomes in a separate cohort of subjects with acute coronary syndromes who were treated with clopidogrel in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON-TIMI). In healthy subjects who were treated with clopidogrel, carriers of at least one CYP2C19 reduced-function allele had a relative reduction of 32.4 % in plasma exposure to the active metabolite of clopidogrel, as compared with noncarriers. Carriers also had an absolute reduction in maximal platelet aggregation in response to clopidogrel that was 9 percentage points less than that seen in noncarriers. Among persons treated with clopidogrel, carriers of a reduced-function CYP2C19 allele had significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition, and a higher rate of major adverse cardiovascular events, including stent thrombosis, than did noncarriers. In another study, among patients with an acute myocardial infarction who were receiving clopidogrel, those carrying CYP2C19 loss-of-function alleles had a higher rate of subsequent cardiovascular events than those who were not (Simon et al. 2009). This effect was particularly marked among the patients undergoing percutaneous coronary intervention.

In the Pharmacogenomics of Antiplatelet Intervention (PAPI) Study (2006–2008), clopidogrel was administered for 7 days to 429 healthy Amish persons and measured response by ex vivo platelet aggregometry (Shuldner et al. Shuldiner et al. 2009). A genome-wide association study was performed followed by genotyping

the loss-of-function CYP 2C19*2 variant (rs4244285). Findings in the PAPI Study were extended by examining the relation of CYP2C19*2 genotype to platelet function and cardiovascular outcomes in an independent sample of 227 patients undergoing percutaneous coronary intervention. Platelet response to clopidogrel was found to be highly heritable. The relation between CYP2C19*2 genotype and platelet aggregation was replicated in clopidogrel-treated patients undergoing coronary intervention. It was concluded that CYP2C19*2 genotype is associated with diminished platelet response to clopidogrel treatment and poorer cardiovascular outcomes.

### Lansoprazole and Cytochrome P450

The acid-inhibitory effect of lansoprazole depends on differences in cytochrome P450 (CYP) 2C19 genotypes. CYP2C19 genotype status, as well as the grade of gastroesophageal reflux disease (GERD) before treatment, is one of the determinants for the success or failure of treatment of GERD with lansoprazole. The low cure rate in patients with the homozygous extensive metabolizer genotype appears to be a result of these patients having the lowest plasma lansoprazole levels among the various genotype groups.

### Glucose-6-Phosphate Dehydrogenase

Phenotypes demonstrating variations in people's response to certain drugs were first discovered in the early 1950s when antimalarial drugs were found to cause hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD, expressed in all of the body's tissues, controls the flow of carbon through the pentose phosphate pathway, produces NADPH for reductive biosynthesis, and maintains oxidation-reduction in the cell to keep glutathione in a reduced state. The absence of reduced glutathione due to G6PD deficiency allows oxidative drugs to oxidize sulfahydroxyl groups of hemoglobin, leading to hemolysis. Currently, over two dozen drugs, including primaquine, sulfones, sulfonamides, nitrofurans, vitamin K analogues, cefotetan, and chloramphenicol, are known to cause hemolytic anemia in G6PD-deficient patients. G6PD deficiency is a sex-linked (chromosome X) recessive trait and a widespread polymorphism, with more than 400 known variants and affecting more than 400 million people worldwide. However, the vast majority of affected individuals are asymptomatic. Only 30 different functional mutations in the gene have been reported, virtually all of which are found in the region of the gene that codes for the protein. All but one are point mutations, with more than 50 % being nucleotide conversions from cytosine to guanine. The consequence of these genetic polymorphisms is low G6PD activity, resulting in reduced glutathione concentrations in erythrocytes and subsequently clinical manifestation of hemolytic anemia following the ingestion of certain drugs.

The prevalence of G6PD deficiency differs among ethnic groups. For instance, males of African and Mediterranean descent more frequently express the trait. In patients with G6PD A, an adenosine-to-guanine substitution at nucleotide 376 (A376G) mutation causes an aspartic acid residue to replace an asparagine residue. There are three different G6PD A (−) variants in one allele. The A376G mutation occurs in all people, but the enzyme deficiency is caused by a second amino acid substitution, usually a G202A mutation, resulting in a valine-to-methionine substitution at codon 68 (Val68Met). Other mutations are Val690Met and Val968Met. In Mediterranean peoples, the most common mutation is a C563T substitution resulting in an amino acid change (Ser188Phe).

Cases of drug-induced hemolytic anemia have also been described in patients treated with cyclosporine, tacrolimus, penicillin, and cefotetan. The risk and severity of hemolysis are thought to be associated with dose, duration of therapy, and other oxidant stresses, such as infection and environmental factors. Because of these confounding factors, genotyping patients for G6PD deficiency is not warranted, since the toxicity is rare and not typically life-threatening and the genotype does not adequately predict the development of hemolytic anemia. For example, some patients with these mutations experience toxicity after drug administration, and others do not. In addition, the treatment for drug-induced oxidative hemolytic anemia is merely cessation of drug administration, with blood transfusion and corticosteroid administration warranted in severe cases.

G6PD deficiency is an example of how genotypic analysis was developed about half a century after the clinical observation was made, and further characterization of the genetic mutation provided no added clinical advantages. Although genetic constitution may be at the core of explaining drug toxicity and efficacy, genotyping may not always directly affect therapy or predict patient outcomes.

## *Pharmacogenetics of Phase II Metabolism*

The N-acetylation of isoniazid was an early example of inherited variation in phase II drug metabolism. Uridine diphosphate-glucuronosyltransferase 1A1 (TATA-box polymorphism) is another. These described in the following sections.

### N-Acetyltransferase

The acetylation polymorphism illustrates another genetic polymorphism of a drug-metabolizing enzyme studied in the early era of pharmacogenetics. N-acetyltransferase (gene, NAT), a phase-II conjugating liver enzyme, catalyzes the N-acetylation (usually deactivation) and O-acetylation (usually activation) of arylamine carcinogens and heterocyclic amines. The slow acetylator phenotype often experiences toxicity from drugs such as isoniazid, sulfonamides, procainamide, and

hydralazine, whereas the fast acetylator phenotype may not respond to isoniazid and hydralazine in the management of tuberculosis and hypertension, respectively. During the development of isoniazid, isoniazid plasma concentrations were observed in a distinct bimodal population after a standard dose. Patients with the highest plasma isoniazid levels were generally slow acetylators and they suffered from peripheral nerve damage, while fast acetylators were not affected. Slow acetylators are also at risk for sulfonamide-induced toxicity and can suffer from idiopathic lupus erythematosus while taking procainamide. The slow acetylator phenotype is an autosomal recessive trait. Studies have shown large variations of the slow acetylator phenotype among ethnic groups: 40–70 % of Caucasians and African-Americans, 10–20 % of Japanese and Canadian Eskimo, more than 80 % of Egyptians, and certain Jewish populations are slow acetylators. In East Asia, the further north the geographic origin of the population, the lower the frequency of the slow acetylator gene. The reason for this trend is unknown, but it has been speculated that differences in dietary habits or the chemical or physical environment may be contributing factors.

Allelic variation at the NAT2 gene locus accounts for the polymorphism seen with acetylation of substrate drugs. There are 27 NAT2 alleles that have been reported. NAT2 is an unusual gene because it consists of open-reading frames (i.e., protein-coding regions) with no introns. Most variant NAT2 alleles involve two or three point mutations. Currently, the importance of these variants in NAT2 is most studied for their association with a modestly increased risk for cancers, possibly because of prolonged exposure of the body to chemicals, drugs, or metabolites compared with fast acetylators. Impaired isoniazid metabolism has been associated with point mutations in NAT2 in a small Japanese population but there is a need for large population studies to establish clearly the relationship between the NAT2 genotype and isoniazid acetylation. It might still take more time to establish the clinical utility of NAT2 genotype analysis to independently predict isoniazid acetylation. However, genotype NAT2 mutations could be an addition to the traditional therapeutic drug monitoring for isoniazid in the near future. Other drugs metabolized by NAT2 are hydralazine and procainamide.

### Uridine Diphosphate-Glucuronosyltransferase

Uridine diphosphate-glucuronosyltransferase 1A1 (TATA-box polymorphism) has a frequency of approximately 10 % among whites and approximately 1 in 2,500 Asians. It is involved in the metabolism of bilirubin and polymorphism in UDG1A1 gene is associated with Gilbert's syndrome (hyperbilirubinemia). Polymorphism also enhances the effect of irinotecan, an antitumor agent approved for use in patients with metastatic colorectal cancer. Its active metabolite, SN-38, is glucuronidated by UGT1A1. Patients with low UGT1A1 activity, such as those with Gilbert's syndrome, may be at an increased risk for irinotecan toxicity.

### *Measurement of CYP Isoforms*

A number of well characterized CYP substrates and inhibitors have been identified that allow precise measurements of individual CYP isoforms. Their use, alone or in combination, facilitates the phenotype characterization of hepatocytes in vitro and in vivo. Two procedures are used for in vitro investigation of the metabolic profile of a drug: incubation with microsomes and incubation with metabolically competent cells. The major limitation of microsomes is that they express phase I activities, but only part of phase II activities, and can only be used for short incubation times. When intact cells are used, gene expression, metabolic pathways, cofactors/enzymes and plasma membrane are largely preserved, but fully differentiated cells such as primary cultured hepatocytes need to be used, since hepatoma cell lines have only very low and partial CYP expression. CYP-engineered cells or their microsomes ('supersomes') have made the identification of the CYPs involved in the metabolism of a drug candidate straightforward and easier.

Inhibition of CYP is an undesirable feature for a drug candidate, and needs to be addressed by examining whether the drug candidate inhibits the metabolism of other compounds or whether other compounds inhibit the metabolism of the drug candidate. Such experiments can be conducted both with microsomes and in cells. The major limitation of microsomes is that inhibition parameters may not accurately reflect the situation in vivo, since the contribution of drug transport is not considered. The best picture of a potential drug-drug interaction can be obtained in metabolically competent hepatocytes. Screening of CYP inducers cannot be done in microsomes. It requires the use of a cellular system fully capable of transcribing and translating CYP genes, and can be monitored in vitro as an increase in enzyme mRNA or activity. Human hepatocytes in primary culture respond well to enzyme inducers during the first few days; this ability is lost thereafter. Rat hepatocytes are much less stable and soon become unresponsive to inducers. Hepatoma cell lines respond poorly to inducers, although the induction of a few isoenzymes has been reported. Primary cultured hepatocytes are still the unique in vitro model that allows global examination of the inductive potential of a drug. However, they are not suitable for high-throughput screening. Genetically manipulated cell lines that express enzymes and respond to inducers would be more suitable for this purpose as an alternative to the use of human hepatocytes.

Polyclonal or monoclonal antibodies raised against CYP isoforms are useful for identification and semiquantitative measurement of the CYP protein. Antibodies can be easily generated by immunization with pure protein isolated from the liver or from cDNA-directed expression systems. Several antibodies against human and animal CYPs are available commercially (http://www.antibodyresource.com/). Inhibiting antibodies can be used for the identification of CYPs involved in the metabolism of a particular compound.

## *Polymorphism of Drug Transporters*

Transporters are involved in the transport of proteins, peptides, amino acids, ions and certain drugs. Transport proteins have an important role in regulating the absorption, distribution, and excretion of many medications. Membrane transporters are encoded by numerous genes. Disorders associated with defects in solute transporters, such as severe diarrhea in glucose/galactose malabsorption and primary bile acid malabsorption may be associated with pronounced general changes in drug absorption. Several investigations are aimed at clarifying the role of transporters in drug absorption, disposition, and targeting.

ABC (ATP-binding Cassette) transporter super family is widely distributed in all living organisms examined to date. It consists of eight subfamilies encoded by genes on different chromosomes. One of these is P-glycoprotein, also called multidrug resistance protein (MDR-1), which serves as a transporter that extrudes numerous drugs out of cells. A variant form of MDR-1 has been associated with low MDR-1 expression and altered drug distribution, resulting in enhanced digoxin plasma levels and suggesting broad implications for drug disposition. An overview of polymorphisms of ABC drug transporters as well as their phenotypical consequences and pharmacological implications has been presented elsewhere.

Another important gene family is the biogenic amine transporters, which regulate neurotransmitter levels in synaptic transmission, with a number of documented variants that may affect function. These transporters are the direct target receptors for numerous drugs, including antidepressants and cocaine. Allelic variations, in particular of the serotonin transporter, are associated with the modulation of complex behavior and may play a significant role in therapy with specific serotonin transporter inhibitors.

## *Genetic Variation in Drug Targets*

Drug targets (e.g. receptors) can have a profound effect on drug efficacy, with over 25 examples already identified. Variation in neurotransmitter receptors can also be the cause of treatment failure. The β2-adrenoreceptor (coded by the ADRB2 gene) illustrates another link between genetic polymorphisms in drug targets and clinical responses. Genetic polymorphism of the β2-adrenoreceptor can alter the process of signal transduction by these receptors. Polymorphisms in drug target genes that can influence drug response are listed in Table 4.5.

### Polymorphisms of Kinase Genes

Kinases are central players in cell biology and disease. Protein kinases are coded by more than 2,000 genes and thus constitute the largest single enzyme family in the human genome. Kinases are important drug targets. Agencourt Bioscience

**Table 4.5** Polymorphisms in drug target genes that can influence drug response

| Gene or gene product | Drug | Effects |
|---|---|---|
| ACE | ACE inhibitors | Renoprotective effects, blood-pressure reduction, reduction in left ventricular mass, endothelial function |
| | Fluvastatin | Reductions in low-density lipoprotein cholesterol and apolipoprotein B Progression or regression of coronary atherosclerosis |
| Arachidonate 5-lipoxygenase | Leukotriene inhibitors | Improvement in $FEV_1$ |
| $\beta_2$ -Adrenergic receptor | $\beta_2$ agonists | Bronchodilatation, susceptibility to agonist-induced desensitization, cardiovascular effects |
| Bradykinin B2 receptor | ACE inhibitors | ACE-inhibitor–induced cough |
| Dopamine receptors (D2, D3, D4) | Antipsychotics | Antipsychotic response (D2, D3, D4), antipsychotic-induced tardive dyskinesia (D3), antipsychotic-induced acute akathisia (D3) |
| Estrogen receptor | Conjugated estrogens Hormone-replacement | Increase in bone mineral density Increase in high-density lipoprotein cholesterol |
| Glycoprotein IIIa subunit of glycoprotein IIb/IIIa | Aspirin or glycoprotein IIb/IIIa inhibitors | Antiplatelet effect |
| Serotonin (5-HT) transporter | Antidepressants | 5-Hydroxytryptamine neurotransmission, antidepressant response |
| Tyrosine kinase | Imatinib mesylate (Gleevec) for chronic myeloid leukemia | A mutation in the Abl kinase domain of the Bcr-Abl gene may produce drug-resistance |



Corporation offers a kinase SNP discovery program for customized polymorphism mapping of human kinase genes. Amplicon modeling, primer design and assay validation have been established for over 1,600 amplicons within 92 different kinase genes. Assays have been extensively optimized to provide high pass rates, low background, and informative results in GC rich regions. Kinase mutation mapping can be used to pinpoint responder populations and facilitate the development of personalized medicine.

## *Effect of Genetic Polymorphisms on Disease Response to Drugs*

Genetic polymorphism of genes and gene products may influence the disease-modifying effects of drugs. DMET™ Plus biomarker panel (Affymetrix) enables genotyping of the largest and most comprehensive set of key functional drug

**Table 4.6** Effect of genetic polymorphisms on disease response to drugs

| Gene or gene product | Drug | Influence of polymorphism on disease response to drug |
|---|---|---|
| Adducin | Diuretics | Decreased myocardial infarction in hypertensive patients |
| Apolipoprotein E (APOE) | Statins | Reduction of progression of atherosclerosis and enhanced survival |
| Cholesterol ester transfer protein (CETP) | Statins | Slowing of progression of atherosclerosis |
| Gs protein α | β-blockers (e.g., metoprolol) | Decreased antihypertensive effect |
| Methylguanine methyl transferase (MGMT) | Carmustine | Enhanced response of glioblastoma to carmustine |
| *Parkin* | Levodopa | Clinical improvement in Parkinson's disease |
| Serotonin transporter (5-HTT) | Antidepressants (e.g., fluoxetine) | Decreased clozapine effects, antidepressant response |
| Stromelysin-1 | Statins | Reduction in cardiovascular events and repeated angioplasty |



metabolism alleles within a single panel. The DMET Plus biomarker panel contains biomarkers in all FDA-validated genes and covers more than 90 % of the current ADME Core biomarkers as defined by the PharmaADME group. It offers 1,936 high value, biologically relevant markers in 225 drug metabolism enzyme, transporter, and transferase genes. Together these features make the Affymetrix DMET panel the most comprehensive one targeting all functional variants within drug metabolism genes.

Some examples of genetic polymorphisms are shown in Table 4.6. Such information is useful in identifying the responders to drugs and is discussed further in subsequent chapters.

## *Ethnic Differences in Drug Metabolism*

Ethnic differences in drug metabolism are well documented for a number of drugs. The molecular mechanisms responsible for ethnic differences in drug metabolism have been partly clarified because of the advances in molecular biology. Genotype analysis indicates a different frequency for the mutant alleles in different ethnic populations, which results in variations in the frequency of subjects who are homozygous for the mutant allele among the extensive metabolizers in different ethnic populations. Ethnic differences in drug metabolism may result from differences in distribution of a polymorphic trait and mutations, which code for enzymes with abnormal activity which occur with altered frequency in different ethnic groups.

Several studies have shown ethnic differences in drug metabolism mediated by CYP2D6 or CYP2C19 have been summarized elsewhere. In most western populations, 93 % are normal or efficient metabolizers (EM), 7 % are poor metabolizers (PM), and less than 1 % are ultrarapid metabolizers (UM) of CYP2D6. In contrast to the Caucasians, only 1 % of the Orientals are PMs. PMs have a metabolic ration (MR) greater than 12.6 and are homozygous for mutations. About 4 % of the Caucasians are PMs of CYP2C19 as compared with about 20 % of the Orientals. One allele ($m_1$) accounts for 75 % of PMs and Orientals have an additional unique allele ($m_2$) accounting for 25 % of PMs. There is risk of adverse effects in PMs and UMs due to abnormal serum levels of the drug. Ethnic factors, therefore, are an important consideration in individualization of therapy.

There are major differences between ethnic groups in the frequency of CYP3A5 expression. For example, 30 % of Caucasians express CYP3A5 and more than 50 % of African Americans express CYP3A5. Liver tissue from Caucasian and African Americans carrying at least one CYP3A5*1 allele contains three times more CYP3A than that from other individuals. The metabolism of a model drug (midazolam) proceeded 2.5 times faster in Caucasians and 2.2 times faster in African Americans with at least one CYP3A5*1 allele compared with metabolism in individuals homozygous for CYP3A5*3. Thus CYP3A5 may be the most important contributor to interracial differences in CYP3A dependent drug clearance and response to many medicines.

## *Gender Differences in Pharmacogenetics*

There are gender-related differences in pharmacokinetics, which may be related to pharmacogenetic differences in to drug-metabolizing enzymes. Men seem to have a higher activity relative to women for the cytochrome P450 (CYP) isoenzymes CYP1A2 and potentially CYP2E1, for the drug efflux transporter P-glycoprotein, and for some isoforms of glucuronosyltransferases and sulfotransferases. Women were suggested to have a higher CYP2D6 activity. No major gender-specific differences seem to exist for CYP2C19 and CYP3A. The often-described higher hepatic clearance in women compared with men for substrates of CYP3A and P-glycoprotein, such as erythromycin and verapamil, may be explained by increased intrahepatocellular substrate availability due to lower hepatic P-glycoprotein activity in women relative to men. Other gender differences in pharmacokinetics may be due to fluctuations in hormone levels in women with menstruation and pregnancy.

For a few drugs, e.g. verapamil, beta-blockers and selective serotonin reuptake inhibitors, gender-related differences in pharmacokinetics have been shown to result in different pharmacological responses, but their clinical relevance remains unproven. Moreover, development of diseases such as heart disease and cancer may affect women differently from men. There is no data to support the efficacy of statins in preventing heart attacks and stroke in women with hypercholesterolemia, partly because there have not been adequate representation of women in clinical

trials as compared to men. Use of statins in women is associated with a higher rate of complications such as myositis and cognitive impairment.

Statin therapy in women without cardiovascular disease is controversial, given the insufficient evidence of benefit. Sex-specific outcomes were analyzed in the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) and the results with prior trials were synthesized (Mora et al. 2010). Participants included 6,800 women and 11, 000 men with high-sensitivity C-reactive protein and low-density lipoprotein cholesterol randomized to rosuvastatin versus placebo. Meta-analysis studies were randomized placebo-controlled statin trials with predominantly or exclusively primary prevention in women and sex-specific outcomes. This study demonstrated that in primary prevention rosuvastatin reduced cardiovascular disease events in women with a relative risk reduction similar to that in men, a finding supported by meta-analysis of primary prevention statin trials.

## *Role of Pharmacogenetics in Drug Safety*

Variability in drug response among patients is multifactorial, including environmental, genetic, and disease determinants that affect the disposition of the drug. Individual variation in response to drugs is a substantial clinical problem. Such variations include failure to respond to a drug, adverse drug reactions (ADRs) and drug-drug interactions when several drugs are taken concomitantly.

### Adverse Drug Reactions

Susceptibility to ADRs varies with genetic make-up, age, sex, physiology, exogenous factors, and disease state. The clinical consequences of ADRs range from patient discomfort through serious clinical illness to the occasional fatality. Some facts about ADRs are:

- There are 2.2 million hospitalizations due to ADRs per year in the US Of these approximately 100,000 die.
- Fatal ADRs are the fourth leading cause of death in the US.
- Twenty percent of all new drugs were eventually reported to be associated with serious adverse reactions that were not known at the time of approval.
- ADRs are a serious problem in infants and young children.
- ADRs are the biggest problem in the elderly – the fastest growing segment of the population in the US.
- The cost of managing adverse drug reactions exceeds $4 billion per annum in the US.
- Ethnic group may act as a marker for underlying genetic or environmental differences in the susceptibility to ADRs, e.g. during treatment with angiotensin converting enzymes and thrombolytic drugs.

### Adverse Drug Reactions in Children

The problem of ADRs in children is being increasingly recognized, and they differ from adult reactions in frequency, nature, and severity. Infants and young children, when exposed to some drugs such as anticholinergic agents, are more likely than adults to develop ADRs, but may also be less susceptible to toxic reactions to other drugs. ADRs in children caused by drugs of abuse are a major problem in the US. Children may be exposed to these drugs through in utero exposure during pregnancy, through breast feeding, and through exposure during adolescence. These ADRs can include effects on the nervous system, cognitive problems, cardiovascular anomalies, and, in the case of second-hand tobacco smoke, an increased risk for sudden infant death syndrome, acute respiratory infections, asthma, middle-ear disease, and multiple sclerosis in children.

The NIH has previously funded research that includes use of genomics, proteomics, and transcriptomics technologies in the discovery and identification of toxicity biomarkers; use of metabolomics alone or in combination with other technology to identify and characterize novel toxicity-associated drug metabolites and unraveling of novel ADR mechanisms; genomic studies that may identify animals that develop idiosyncratic reactions similar to humans; using genomics to define patterns of genes association with pediatric ADRs; placental genomics, proteomics, and biomarker identification to understand ADRs; the role of epigenetic factors to explain or predict developmental differences in the expression of ADRs; and other studies.

### Adverse Drug Reactions Related to Toxicity of Chemotherapy

Neurotoxicity and myelotoxicity are well known adverse reactions of chemotherapy in cancer patients. Scientists at the NCI have evaluated the relationships between ABCB1 (P-glycoprotein, MDR1) polymorphisms and paclitaxel (Taxol)-induced toxicity and pharmacokinetics (Sissung et al. 2006). Patients carrying two reference alleles for the ABCB1 3435C>T polymorphism trended toward a reduced risk to develop neuropathy as compared to patients carrying at least one variant allele. Additionally, patients who were homozygous variant at the 2677 and 3435 loci had a significantly greater percent decrease in absolute neutrophil count at nadir. Neither polymorphism correlated with paclitaxel pharmacokinetics. This pilot study suggests that paclitaxel-induced neuropathy and neutropenia might be linked to inherited variants of ABCB1 through a mechanism that is unrelated to altered plasma pharmacokinetics. NCI is seeking commercial partnering to market a test based on ABCB1 genotyping to predict toxicity of chemotherapy in individual patients.

**Genetically Determined Adverse Drug Reactions**

One reason for the high incidence of serious and fatal ADRs is that the existing drug development does not incorporate genetic variability in pharmacokinetics and pharmacodynamics of new drug candidates. Polymorphisms in the genes that code for drug-metabolizing enzymes, drug transporters, drug receptors, and ion channels can affect an individual's risk of having an adverse drug reaction, or can alter the efficacy of drug treatment in that individual. Mutant alleles at a single gene locus are the best studied individual risk factors for adverse drug reactions, and include many genes coding for drug-metabolizing enzymes. These genetic polymorphisms of drug metabolism produce the phenotypes of "poor metabolizers" or "ultrarapid metabolizers" of numerous drugs. Together, such phenotypes make up a substantial proportion of the population. Genetic aberrations associated with adverse reactions are of two types. The vast majority arise from classical polymorphism in which the abnormal gene has a prevalence of more than 1 % in the general population. Toxicity is likely to be related to blood drug concentration and, by implication, to target organ concentration as a result of impaired metabolism. The other type is rare and only 1 in 10,000 to 1 in 100,000 persons may be affected. Most idiosyncratic drug reactions fall into the latter category. Mutant alleles at a single gene locus are the best studied individual risk factors for adverse drug reactions, including the genes for N-acetyltransferases, thiopurine methyltransferase, dihydropyrimidine dehydrogenase, and cytochrome P450. However, pharmacogenetic factors rarely act alone; rather they produce a phenotype in concert with other variant genes such as those for receptors and with environmental factors such as cigarette smoking. Examples of adverse reactions with a pharmacogenetic basis are shown in Table 4.7 and this can form the basis of practice of genotyping prior to decision to use a drug that might produce serious adverse reactions.

Most idiosyncratic drug reactions are unpredictable and because of their rarity my not show up in patients during clinical trials with a few thousand patients. They may first surface when the drug has been taken by hundreds of thousands of patients in the post-marketing phase. Pharmacogenetics, by individualizing treatment to patients for whom it is safe, provides a rational framework to minimize the uncertainty in outcome of drug therapy and clinical trials and thereby should significantly reduce the risk of drug toxicity.

Topiramate, an anticonvulsant medication, is an efficacious treatment for alcohol dependence. A study has examined three SNPs of the glutamate receptor GluR5 gene (GRIK1) as predictors of topiramate-induced side effects in the context of a laboratory study of topiramate (Ray et al. 2009). Analyses revealed that an SNP in intron 9 of the GRIK1 gene (rs2832407) was associated with the severity of topiramate-induced side effects and with serum levels of topiramate. Genes underlying glutamatergic neurotransmission, such as the GRIK1 gene, may help predict heterogeneity in topiramate-induced side effects. Future studies in larger samples are needed to more fully establish these preliminary findings.

**Table 4.7** Examples of genetically determined adverse reactions to drugs

| Drug | Adverse reaction | Underlying gene/mutation |
|---|---|---|
| 6-mercaptopurine | Myelotoxicity, pancytopenia | Thiopurine methyltransferase (TMPT) |
| Azathioprine | Carcinogenicity | |
| β2-agonists | Increased airway reactivity | β2-receptor |
| Debrisoquin | Hypersensitivity | CYP2D6 |
| Fluorouracil | Increased neurotoxicity | Dihydropyrimidine dehydrogenase |
| Fructose | Intolerance | Aldolase B |
| Inhalation anesthetics | Malignant hyperthermia | Ryanodine receptor |
| Irinotecan | Diarrhea | Uridine diphosphate glucuronosyl transferase 1A1 |
| | Myelosuppression | |
| Primaquine | Hypersensitivity: favism | Glucose-6-phosphate dehydrogenase |
| Proton pump inhibitors | Reduced efficacy in curing ulcers | CYP2C19 |
| Sulfonal | Porphyria | Porphobilinogen deaminase |
| Suxamethonium | Hypersensitivity | Pseudocholinesterase |
| Typical antipsychotic drugs | Extrapyramidal effects, confusion | Dopamine D3 receptor |
| | Cardiotoxicity | 5-$HT_{2C}$ receptor |
| Warfarin anticoagulation | Reduced clearance of the drug leading to hemorrhage | CYP2C9 |
| | Interaction with NSAIDs | |
| | Interaction with Tramadol | |



Other genetic biomarkers that can be used to predict ADRs are (Ingelman-Sundberg 2008):

- UGT1A1*28 to predict ADRs to irinotecan in 30–40 % of cases.
- CYP2C9 and VKORC1 to predict ADRs to tricyclic antidepressants in 5–7 % of cases.
- HLA-B*5701 to predict ADRs to abacavir in 5–8 % of cases.
- HLA-B*1502 to predict ADRs to carbamazepine in 10 % of cases.
- HLA-DRB1*07 and DQA1*02 to predict ADRs to ximelagatran in 5–7 % of cases.

In some situations, genotyping information may enable the avoidance of use of a drug in certain patients prone to serious adverse reactions such as azathioprine in patients with TMPT deficiency and malignant hyperthermia in patients undergoing anesthesia. In other situations, it may help in the adjustment of dose of the drug such as in warfarin therapy.

### Malignant Hyperthermia

Malignant hyperthermia (MH) is a pharmacogenetic clinical syndrome that manifests as a hypermetabolic crisis when a susceptible individual is exposed to an anesthetic triggering agent. Clinical signs include unexplained elevation of end-tidal

carbon dioxide, muscle rigidity, acidosis, tachycardia, tachypnea, hyperthermia, and evidence of rhabdomyolysis. This process is a result of an abnormally increased release of calcium from the sarcoplasmic reticulum, which is often caused by an inherited mutation in the gene for the ryanodine receptor (RYR1) that resides in the membrane of the sarcoplasmic reticulum. The gold standard for determination of MH susceptibility is the caffeine-halothane contracture test. However, it is invasive, requiring skeletal muscle biopsy and is not widely available. Researchers have begun to map mutations within the ryanodine receptor gene (chromosome 19q13.1) responsible for conferring MH susceptibility. Ryanodine receptor mutations are found in at least 25 % of known MH susceptible individuals in North America. Mutation analysis is now available commercially and is expected to play an integral role in the diagnosis of MH susceptibility in the future.

### Pharmacogenetics of Clozapine-Induced Agranulocytosis

Clozapine has long been accepted as one of the most effective medications for treating schizophrenia but has had limited utilization due to the risk of inducing agranulocytosis, a life-threatening decrease of white blood cells that requires frequent blood testing of patients. In 2004, CARING (Clozapine and Agranulocytosis Relationships Investigated by Genetics) study, reported the discovery of genetic biomarkers that predict who is at risk of developing clozapine-induced agranulocytosis. This raised the hope for a one-time genetic test may obviate the need for continuous blood monitoring for the majority of clozapine- treated patients. These findings have uncovered new clues to the underlying biological and physiologic mechanisms of drug-induced agranulocytosis and provide a starting point for elucidating a common mechanism across drugs from different classes that carry this rare but devastating side effect. The sensitivity and selectivity of these biomarkers could support further development of a diagnostic test. One of the associations identified in the HLA (Human Leukocyte Antigen) complex has been previously reported to be associated with clozapine-induced agranulocytosis.

However, no genetic test is currently available for clozapine-induced agranulocytosis. The proposed test of HLA-DQB1 6672G>C has high specificity but low sensitivity, and fails to reduce the agranulocytosis risk in the LR group to a point that monitoring could be reduced or ceased (Verbelen et al. 2014). Candidate gene studies have failed to identify a strong, replicated genetic variant that substantially increases risk of clozapine-induced agranulocytosis. The first genome-wide association study of clozapine-induced agranulocytosis detected significant associations at two HLA amino acids (Goldstein et al. 2014). At least one further study of development of pharmacogenomic biomarkers for schizophrenia, CRESTAR, is in progress (http://www.crestar-project.eu/). Combined analysis of such studies may identify associated genetic variants that can be rapidly translated to clinical practice.

**Role of Pharmacogenetics in Warfarin Therapy**

Warfarin (Coumadin) is the most commonly prescribed oral anticoagulant for the treatment and prevention of thromboembolic events. Approximately two million patients in the US are initiated on warfarin therapy each year. The correct maintenance dose of warfarin for a given patient is difficult to predict, the drug carries a high risk of toxicity, and variability among patients means that the safe dose range differs widely between individuals. Currently complications of warfarin therapy account for 10.5 % of the hospital admissions for adverse drug reactions, the second most common reason for patients to go to the emergency room. Recent pharmacogenetic studies indicate that the routine incorporation of genetic testing into warfarin therapy protocols could substantially ease both the financial and health risks currently associated with this treatment (Reynolds et al. 2007). Genotype knowledge of the CYP2C9 variant alleles may help the clinician to individualize warfarin therapy with the ultimate goals of shortening the initial period of induction therapy, reaching a stable maintenance dose earlier, and minimizing bleeding complications in patients who are high responders and need lower warfarin doses. In 2007, the FDA made the following recommendations:

- Lower doses of warfarin should be used in patients with genetic variations in CYP2C9 and VKORC1 genes.
- Genotyping patients in the induction phase of warfarin therapy would reduce adverse events and improve therapy achievement of stable International Normalized Ratio.
- Existing evidence of the influence of CYP2C9 and VKORC1 genotypes warrants re-labeling of warfarin to include genomic and test information.

The labeling update is a milestone that brings personalized medicine to the mainstream. However, the FDA further emphasized that this labeling update is not a directive to physicians to use genetic tests for warfarin therapy. That kind of a label will have to wait for outcomes data. To this end, there are numerous studies currently ongoing looking at outcomes when genetic tests are incorporated into warfarin treatment. The Harvard Partners Center for Genetics and Genomics, Medco and the Mayo Clinic, Clinical Data and PharmaCare, and the University of Utah under the Critical Path Initiative, are all researching the clinical utility of pharmacogenetics-based warfarin dosing. FDA cleared Verigene Warfarin Metabolism Nucleic Acid Test (Nanosphere Inc), which detects variants of CYP2C9 and VKORC1 genes, responsible for sensitivity to warfarin. The FDA also cleared Verigene® F5/F2/MTHFR nucleic acid test, which detects disease-associated gene mutations that can contribute to blood coagulation disorders and difficulties metabolizing folate (vitamin B12). Mutations in three specific genes can increase an individual's risk for dangerous blood clots and their leading complication, and is an indication for warfarin therapy. The use of a pharmacogenetic algorithm for estimating the appropriate initial dose of warfarin produces recommendations that are significantly closer to the required stable therapeutic dose than those derived from a clinical algorithm or a fixed-dose approach (The International Warfarin Pharmacogenetics Consortium 2009).

A genome-wide association study found gene polymorphisms that affect the anticoagulant effect of warfarin (Takeuchi et al. 2009). The study confirmed VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. They also thoroughly investigated copy number variations, haplotypes, and imputed SNPs, but found no additional highly significant warfarin associations. These results provide justification for conducting large-scale trials assessing patient benefit from genotype-based forecasting of warfarin dose. A multicenter, randomized, controlled trial of warfarin involving patients with atrial fibrillation or venous thromboembolism in whom genotyping for CYP2C9*2, CYP2C9*3, and VKORC1 (−1639G → A) was performed with the use of a POC test (Pirmohamed et al. 2013). Results showed that pharmacogenetic-based dosing was associated with a higher percentage of time in the therapeutic INR range than was standard dosing during the initiation of warfarin therapy. An individualized dose forecasting based on a patient's genetic makeup at VKORC1, CYP2C9 and possibly CYP4F2 could provide state-of-the-art clinical benchmarks for warfarin use during the foreseeable future.

## Role of Pharmacogenetics in Antiplatelet Therapy

The antiplatelet agent clopidogrel (Plavix) is used in the management of cardiovascular disease and stroke, but genetic mutations may reduce the effect of this drug. The reduced-function CYP2C19 allele is present in around 30 % of people of European ancestry and more than 40 % of those of African or Asian ancestry. Persons with these gene variants carry double or triple the risk of death, myocardial infarction or stroke, compared with people with the normal metabolism alleles.

It has been suggested that clopidogrel may be less effective in reducing the rate of cardiovascular events among persons who are carriers of loss-of-function CYP2C19 alleles that are associated with reduced conversion of clopidogrel to its active metabolite. Lack of anticipated platelet response may be due to development of resistance to clopidogrel, which is likely due to a polymorphism of the CYP2C19 gene (Ford 2009). One study found that determination of ABCB1 genotype in addition to CYP2C19 enables better prediction of clopidogrel nonresponsiveness (Momary et al. 2010). The FDA has changed clopidogrel's prescribing information to highlight the impact of CYP2C19 genotype on clopidogrel pharmacokinetics, pharmacodynamics and clinical response (Ellis et al. 2009). However, another study in patients with acute coronary syndromes or atrial fibrillation has shown that the effect of clopidogrel as compared with placebo is consistent, irrespective of CYP2C19 loss-of-function carrier status (Paré et al. 2010).

In 2009, Scripps Health was the first health system in the US to deploy Quest/Scripps CYP2C19 genetic testing service for coronary stent patients receiving Plavix. As of March 2011, Quest is collaborating with Scripps Translational Science Institute to develop the Plavix-Response test for 3 M Integrated Cycler to personalize treatment with Plavix based on a patient's CYP2C19 genotype in less than 1 h.

Spartan Bioscience launched a DNA testing system in 2010 for the CYP2C19*2 mutation that guides how patients metabolize Plavix. It is currently in a clinical trial

in Canada, and the data from the pharmacogenomic study will help support regulatory filings for the combined use of the test with Plavix.

INFINITI CYP2C19 Assay (AutoGenomics) is approved by the FDA to clinicians in determining therapeutic strategy for drugs that are metabolized by the CYP450 2C19 gene product. Although several studies have shown that carriers of CYP2C19 alleles do not respond to Plavix as well as non-carriers, the FDA has updated the drug's label to notify physicians of the role of these alleles in Plavix metabolism, the assay's label specifically notes that "the INFINITI CYP2C19 Assay is not intended to be used to predict drug response or non-response".

The *17 variant CYP2C19 gene mutation adds more complexity to the evaluation of clopidogrel therapy because it results in an enzyme that is classified as gain-of-function, and the patient is considered an ultra-rapid metabolizer (Sibbing et al. 2010). In patients undergoing coronary stent placement, a single *17 gene variant resulted in about a twofold increase in the incidence in bleeding within 30 days following stent placement. The incidence of bleeding was 2.5 % in patients without *17, but 4 % in patients with a single *17 variant. Patients with the double *17/*17 genotype exhibited a dose-response fourfold increase in bleeding (8 %). The most striking finding was that among the patients tested, 35 % had the single *17 variant and another 5 % had the *17/*17 genotype.

Clopidogrel GenoSTAT test (Iverson Genetics) identifies the genetic (CYP2C19) cause of an individual's resistance to the drug, detects mutation of the P450-2C19 genotypes (*2, *3, *4, *5, *6, *7,* 8 & *17) and determines if a patient should be prescribed clopidogrel as a therapy. The test offers eight different variants.

According to the recommendation from the American College of Cardiology on genotyping for clopidogrel, genotyping may be considered for identifying poor metabolizers in "moderate to high risk patients" as alternate therapies are available (Holmes et al. 2010). In agreement with these recommendations, another publication pointed out that with stent thrombosis rates in carriers of CYP2C19 as high as 11 %, mortality associated with such events close to 50 %, and a near twofold increase in the risk of bleeding in CYP2C19*17 carriers, it makes clinical sense to implement all potential interventions to help prevent the catastrophic outcomes of stent thrombosis and death, rather than wait years for results from future prospective trials to be initiated (The Case for Routine Genotyping in Dual-Antiplatelet Therapy (Damani and Topol 2010)).

Although the FDA recommended that CYP2C19 genotyping be considered prior to prescribing clopidogrel, researchers from the UK have published a systematic review and meta-analysis that casts doubt on the effectiveness of such testing (Holmes et al. 2011). They showed that although there was an association between the CYP2C19 genotype and clopidogrel responsiveness, there was no significant association of genotype with cardiovascular events. In response to this publication, cardiologists from Scripps Health as well as colleagues from Vanderbilt University and Hôpital Pitié-Salpetrière in Paris pointed out what they see as flaws in the review analysis. They especially take issue with a metaanalysis of stent thrombosis associated with CYP2C19 loss-of-function alleles, in which the UK researchers concluded "an absolute increase of 14 stent thromboses per 1,000 individuals".

With >1 million patients undergoing coronary stenting per year in the US, this extrapolates to >14,000 stent thrombosis events per year! Moreover, the meta-analysis did not test for heterogeneity among patients who underwent stenting versus those who were medically treated. They pointed out that prior pharmacogenomic studies on clopidogrel have shown CYP2C19 loss-of-function variants to be important in coronary stenting patients and does not show up in those medically treated. It is obvious that a metal implant in a coronary artery would pose a particular vulnerability to inadequate platelet suppression.

### Role of Pharmacogenetics in Carbamazepine Therapy

Carbamazepine is responsible for severe ADRs such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) and there is a high incidence of these ADRs in Taiwan compared to other countries. In 2007, Taiwan's Department of Health updated the label for the anticonvulsant drug carbamazepine (CBZ) to warn patients of a genetic link to potentially serious side effects of CBZ and recommends testing patients for predisposition to these ADRs. A series of retrospective studies has shown that the human leukocyte antigen (HLA)-B*1502 marker, which is present in about 5 % of the Taiwanese population has a very strong association with these serious ADRs. The updated label notes this risk and warns that a patient who carries the HLA-B*1502 gene will have at least 193 times higher risk of developing ADR than a patient who is not a HLA-B*1502 carrier (Lee et al. 2010). Identification of subjects carrying the HLA-B*1502 allele and the avoidance of carbamazepine therapy in these subjects was strongly associated with a decrease in the incidence of carbamazepine-induced SJS–TEN (Chen et al. 2011).

The presence of the HLA-A*3101 allele was associated with carbamazepine-induced hypersensitivity reactions among subjects of Northern European ancestry. The presence of the allele increased the risk from 5.0 % to 26.0 %, whereas its absence reduced the risk from 5.0 % to 3.8 % (McCormack et al. 2011).

The strong association between CBZ and HLA-B*1502 has prompted the FDA to update the label for CBZ to include genetic information and to recommend genetic testing before prescribing CBZ. Patients with Asian ancestry or who are from regions prevalent in HLA-B*1502 should be screened before CBZ treatment.

### Role of Pharmacogenetics in Statin Therapy

Lowering low-density lipoprotein cholesterol with statin therapy results in substantial reductions in cardiovascular events, and larger reductions in cholesterol may produce larger benefits. In 10–20 % of cases, myopathy occurs in association with statin therapy, especially when the statins are administered at higher doses and with certain other medications and is a reason for discontinuation. Only half of the patients remain on statin therapy after starting to use it. A genome wide association study of patients on simvastatin (Zocor) therapy has identified SNP rs4363657

located within SLCO1B1 on chromosome 12, what is strongly associated with an increased risk of statin-induced myopathy. SLCO1B1 encodes the organic anion-transporting polypeptide OATP1B1, which has been shown to regulate the hepatic uptake of statins. Genotyping these variants may help to achieve the benefits of statin therapy more safely and effectively (SEARCH Collaborative Group 2008). The finding raises hope that a test could be developed to screen patients to find out who is at greatest risk for developing this adverse reaction. SINM PhyzioType System, which is in development, consists of four tests that predict LDL lowering and HDL raising capabilities, myalgia, and creatine kinase activity in response to statins. The goal is to enable clinicians to deploy a genetic decision support system to manage statins, prescribe these drugs on a DNA-guided, personalized basis, and effectively lower the risk of cardiovascular disease for each patient. Although there is a statin-gene association for myopathy in the case of some statins, the usefulness of this information still needs to be proven (Giorgi et al. 2011).

### FDA Consortium Linking Genetic Biomarkers to Serious Adverse Events

The FDA's has created a consortium with members of the pharmaceutical industry and academia that aims to observe how genetic biomarkers contribute to serious adverse events (SAEs). SAE consortium (SAEC) will be part of the Office of Critical Path Programs. Some people are genetically predisposed to have SAEs to some drugs, and the FDA is of the opinion that it not in its best interests of the patients that the drug manufacturers simply launch these products without putting appropriate information on labels. SAEC also plans to consult the European Agency for the Evaluation of Medicinal Products and other national regulatory bodies for guidance.

Member organizations of the SAEC (http://www.saeconsortium.org/) include Abbott, GlaxoSmithKline, Johnson & Johnson Pharmaceutical Research & Development, Pfizer, Roche, Sanofi-Aventis, Wyeth, Newcastle University, DILIGEN (a UK program that is developing a test to identify patients at high risk of developing drug-induced liver disease), EUDRAGENE (a European academic consortium conducting research on drug-related liver toxicity), Illumina, and Columbia University (New York). The companies are paying $500,000 each to be involved in the consortium. Some pharmaceutical companies are skeptical and will not join as they think that the consortium will have little effect on tracking and avoiding SAEs. The problem is that it will take thousands and thousands of patients to screen in order to validate a particular marker. SAEs, which include hepatotoxicity, rabdomyolysis, and QT prolongation, among others, typically occur in less than one in 1,000 patients and are inherently unpredictable either by preclinical or clinical development. Because of the rarity of such events, the prospect of predicting them by genetic biomarkers is viewed as not only daunting but unlikely. Nevertheless, SAEC is grappling with a central challenge of drug development: the fact that SAEs affecting only a few patients can hold up or prevent the release of a drug that could help many.

The SAEC is not the only federal initiative aimed at improving drug safety. The Critical Path is also linking the Association of Clinical Research Organizations with the Clinical Data Interchange Standards Consortium to form the Clinical Data Acquisition Standards Harmonization project. This group was charged with developing sample case report forms for reporting adverse events according to a NIH summary of a Roadmap steering committee meeting that took place in 2006. According to the summary, the office does cross-cutting coordination and harmonization of all the centers within the FDA. These include the Oncology Biomarker Qualification Initiative, which pairs the FDA with the National Cancer Institute and the Centers for Medicaid and Medicare Services; the Biomarker Consortium, which brings together the FDA, the NIH, and the Pharmaceutical Research & Manufacturers of America. Areas of focus in this effort are bioinformatics and data standards, biomarkers, establishing public-private partnerships, and developing guidance and regulations.

SAEC has released its findings on the genetics of Stevens-Johnson syndrome and has participated in studies identifying variants linked to drug-induced liver injury from the use of the antibiotic flucloxacillin. SAEC collaborates with the HMO (Health Maintenance Organization) Research Network to explore the possibility of using HMO's clinical data to study drug-related serious adverse drug reactions. It is also partnering with researchers at Duke University to look for rare variants corresponding to adverse reactions to the antipsychotic drug clozapine. All of the information SAEC gains from these studies is openly accessible so it may be developed into tests.

SAEC's work is based on the hypothesis the differences in response to drugs by side effects have (in part) a genetic basis, and its research studies examine the impact genetic variation on how individuals respond to a large variety of medicines. Majority of the SAEC's genetic findings have been specific to a given drug versus across multiple drugs. However, a number of cross drug genetic alleles are starting to emerge that may provide important insights into the underlying biology/mechanism of drug induced SAEs (e.g. HLA*5701 or UGT1A1*28). These findings clearly demonstrate an important role for the MHC genomic region (Chromosome 6), in the pathology of immunologically mediated SAEs. They also emphasize the importance of immune regulation genes, in addition to a number of well characterized drug metabolism genes. SAEC's second phase (2010–2015) will develop novel, international clinical networks to deepen an understanding of the genetics of the following SAEs across a diverse range of ethnic populations:

- Acute hypersensitivity syndrome
- Excessive weight gain (associated with class 2 antipsychotic medications)
- Hepatotoxicity
- IBD therapy related SAES (four different phenotypes)
- Nephrotoxicity
- Osteonecrosis of jaw
- Serious skin rash

## *Therapeutic Drug Monitoring, Phenotyping, and Genotyping*

Therapeutic drug monitoring (TDM) has been used for over three decades to investigate variations in drug response but the specific drug metabolism of phenotype may be identified by either phenotyping or genotyping approaches.

### Therapeutic Drug Monitoring

TDM has been used to eliminate variable pharmacokinetics as a source of nonresponsiveness as well as adverse drug reactions. TDM is particularly useful in drugs displaying one or more of the following:

- Steep concentration effect curve and thus narrow therapeutic index
- Delayed clinical effects
- Necessity of dose titration
- Multiple pharmacodynamic mechanisms of action in connection with the different concentrations

Advantages of TDM are:

- Determines the phenotypes of the drug currently in use
- Discovers drug interactions
- Verifies compliance

Limitations of TDM are:

- A steady state is needed
- Possible repetitive monitoring may require multiple blood samples
- Does not predict metabolic capacity

### Phenotyping

Phenotyping is accomplished by administration of a test drug the metabolism of which is known to be dependent solely on the function of a specific drug-metabolizing enzyme followed by measurement of the metabolic ratio, which is the ratio of the drug dose to metabolite measured in serum or urine. Thus it predicts metabolic capacity for a variety of drugs. Phenotyping can reveal defects in overall metabolism of a drug or drug-drug interactions but it has several disadvantages:

- Requires a test drug
- Testing protocol is complicated
- Risk of adverse drug reactions
- Errors in phenotype assignment due to co-administration of drugs
- Confounding effect of the disease

Comprehensive phenotyping is important for understanding disease mechanisms and variations in disease course and response to therapy among patients. SurroMed Inc's phenotyping technology platform provides fundamental information about disease and enables rapid discovery of new and useful biological markers. These biological markers will have utility for better diagnosing and treating disease and developing new and improved therapeutic products.

Metaprobe™ biomarkers (Phenome Sciences) offer an improved approach to identifying a patient's phenotype. Metaprobes measure the capacity of targeted pathways that are instrumental in a disease process or metabolic pathway relevant to the activity of a pharmaceutical. Structurally, Metaprobe biomarkers are small molecules such as amino acids or other compounds that have confirmed safety profiles and can be delivered orally, by injection, or by inhaler. Metaprobes are labeled to quantify pathway capacity by detection of release tags in breath, plasma, or urine. The rate of appearance of the release tag gives a direct and quantitative measurement of the in vivo activity of the targeted pathway, creating a dynamic biomarker of phenotype. Metaprobes are available for over 120 pathways in various stages of active development. For example, metaprobes can provide very sensitive assessment of physiologic response to a known therapeutic that changes internal demand for glutathione. Metaprobe biomarkers have been demonstrated in the following paradigms:

- Identification of a large population with strong efficacy and no significant side effects, allowing smaller, faster trials with higher odds of success
- Characterization of optimal dosage from Phase II trials in order to increase the success rate in phase III trials
- Mechanism confirmation with safety information from first-in-man tests, leading to better phase II study design
- Selection of the best drug candidates from animal studies for clinical development, enhancing drug discovery productivity
- Completion of mechanism-based discovery to understand novel pathways as potential drug targets, enabling effective translation of genomics information into drug creation

Efficient and comprehensive large-scale phenotyping technologies are needed to understand the biological function of genes. This presents a difficult challenge because phenotypes are numerous and diverse, and they can be observed and annotated at the molecular, cellular and organismal level. New technologies and approaches will therefore be required. Efforts to develop new and efficient technologies for assessing cellular phenotypes include the following:

- A phenotypic map can be generated to correspond to any genotypic map. Some genes have only one corresponding phenotype whereas most genes have many corresponding phenotypes.
- The most complete gene annotation is available for simple microbial-cell systems.
- Phenotype microarray technology enables the testing of thousands of phenotypes.

### Genotyping

Genotyping also predicts metabolic capacity but involves identification of defined genetic mutations that give rise to the specific drug metabolism phenotype. These mutations include genetic alterations that lead to overexpression (gene duplication), absence of an active protein (null allele), or production of a mutant protein with diminished catalytic capacity (inactivating allele). Genetic mutations can be screened by molecular diagnostic methods. Advantages of genotyping are:

- Not affected by coadministered medications
- Only one blood sample is needed
- Information acquired has life-long validity

### Genotyping vs Phenotyping

Genotyping has 100 % specificity for detection of impaired metabolizers of CYP2D6 due to genetic reasons but with respect to sensitivity phenotyping is still the preferred method. Phenotype (sensitivity 98 %) provides information on CYP2D6 function, whether it is influenced by either genotype or acquired hepatic disease. Genotyping, on the other hand, provides time invariant information on the individual's metabolizing capacity and it is applied in clinical and epidemiological studies. If therapeutic decisions are based on this information, 10–20 % of poor metabolizes may be wrongly classified as extensive metabolizes. Genotyping is valuable both for individual cases, particularly when a phenotype cannot be established due to concomitant therapy, and for screening of populations in clinical studies.

Phenotype tests have applied successfully in some pharmacogenetics conditions such as malignant hyperthermia, porphyries and glucose-6-phosphate dehydrogenase deficiency. It is likely that more practical genotyping tests would be used in the future and phenotypes would be predicted via genotyping. The traditional phenotype-to-genotype pharmacogenetic research paradigm is reversing direction to create a complementary genotype-to-phenotype flow of information. Examples of genotyping and phenotyping are shown in Table 4.8.

## *Phenomics*

Phenomics is the study of genomics information to better understand the complex relationship between genotype and phenotype. This relationship is frequently nonlinear in nature, which poses a problem for traditional means of genetic study. These traditional methods are not well suited to accommodate the effect of quantitative trait loci or multi-dimensional genetic interactions at work in the determination of most human phenotypes.

**Table 4.8** Examples of genotyping and phenotyping in some diseases

| Disease | Clinical features | Precipitating factors | Phenotyping | Genotyping |
|---|---|---|---|---|
| α1-antitrypsin deficiency (AAT) | Early onset of emphysema and liver failure | Smoking | Plasma α1-antitrypsin concentration | >30 AAT gene mutations on chromosome 14q31-32.3 |
| Congenital adrenal hyperplasia | An autosomal recessive disorder with several clinical manifestations | | Serum 17-hydroxy-progesterone levels | >50 mutations of 21-hydroxylase (CYP21) gene on chromosome 6p21.3 near HLA-B locus |
| Cystic fibrosis | Build-up of thick, sticky mucus in the airways | Liver disease and malabsorption reduces drug availability | Sweat chloride concentration | >1,000 mutations of CFTR gene on chromosome 7q31 |
| Glucose-6-phosphate dehydrogenase deficiency | Growth retardation, hypoglycemia, intravascular hemolysis | Drugs: antimalarials, sulfonamides, quinidine | Absence of ultraviolet-induced fluorescence of erythrocytes | Point mutations of G6PD gene on chromosome Xq28 |



The term 'phenomics' is coined to describe, in anticipation, the new field that is likely to form from the behavioral and other phenotypic analyses designed to obtain a large amount of information on the varying effects of genetic mutations. This will integrate multidisciplinary research, with the goal of understanding the complex phenotypic consequences of genetic mutations at the level of the organism. Hardware and software engineers, as well as behavioral (and other) neuroscientists will co-develop test paradigms and equipment that will enable investigators to cope with the demands set by the increasing number of mutants generated by such techniques as transgenics or chemical mutagenesis. Phenomics will be a crucial approach in academic, as well as industrial, research and could lead to a significant paradigm shift both in the genetic analysis of brain function and in drug development.

The Phenome platform system of DNAPrint Inc will help identify an individual predisposed to develop cancer before the onset of illness so that lifestyles can be altered and/or preventative measures taken. It will be used to identify individuals who are incompatible with certain drug treatments before the drugs are prescribed and damage is done. It will be used to tease out important genetic determinants associated with complex genetic diseases, so that drugs can be developed to target these genes.

### Limitations of Genotype-Phenotype Association Studies

Although genotype-phenotype association studies are seemingly simple, in practice, they are prone to potential difficulties and problems. Plausible biologic context consistent with allele function, low P values, independent replication of an initial study, rigorous phenotypic assessment and genotyping, selection of appropriate and sufficiently large populations, and appropriate statistical analysis are all critical to the confidence that can be placed in a proposed association. Because such criteria are not always met, the risk of false-positive or false-negative errors is always possible. Some of the disparities between genotype and phenotype clarified by metabolomics as described in Chap. 6.

## *Molecular Toxicology in Relation to Personalized Medicines*

The term molecular toxicology covers the use of molecular diagnostic methods for studying the toxic effects of drugs. Toxicology studies are an important part of the drug development process. During preclinical testing, pharmacogenetics methods can be applied to determine drug toxicity at the molecular level during animal studies or to provide an alternative to in vitro/in vivo assays. A number of assays have been developed to assess toxicity, carcinogenicity, and other genetic responses that arise when living cells are exposed to various chemical compounds. Two important categories of molecular toxicology are: toxicogenomics (use of genomic technologies for the study of toxicology) and toxicoproteomics (see Chap. 5). The object of these studies is to detect suitable drug candidates at an early stage of the discovery process and to reduce the number of failures in later stages of drug development.

### Toxicogenomics

Toxicogenomics is the application of genomic technology to toxicology to study how the entire genome is involved in biological responses of organisms exposed to environmental toxicants/stressors. Researchers use toxicogenomic data to determine how human genes respond and interact with each other during different states of health, disease and challenges from toxicants. This discipline is the focus of study of the National Center for Toxicogenomics (Bethesda, MD), a division of the National Institute of Environmental Health Sciences of the National Institutes of Health of USA. Technologies to measure and compare gene expression levels are being increasingly applied to in vitro and in vivo drug toxicology and safety assessment.

Two main technologies for toxicogenomics are those used for measuring gene expression and SNP genotyping. SNPs and other genetic differences have been directly linked to variation in drug metabolism. Various technologies for SNP genotyping have already been described in Chap. 2. Use of microarray technologies for toxicogenomics will be described later in this chapter.

Increasingly, genetic polymorphisms of transporter and receptor systems are also recognized as causing interindividual variation in drug response and drug toxicity. However, pharmacogenetic and toxicogenetic factors rarely act alone; they produce a phenotype in concert with other variant genes and with environmental factors. Environmental factors may affect gene expression in many ways. For instance, numerous drugs induce their own and the metabolism of other xenobiotics by interacting with nuclear receptors such as AhR, PPAR, PXR and CAR. Genomics is providing the information and technology to analyze these complex situations to obtain individual genotypic and gene expression information to assess the risk of toxicity.

### Biomarkers of Drug Toxicity

This topic is discussed in detail in a special report on biomarkers (Jain 2015). Clinical chemistry endpoints for routine animal toxicity testing and clinical trial safety monitoring have been used for over 25 years. Drug-induced damage to the liver is the most common type of toxicity that results in withdrawn of a drug from clinical trials or from further marketing. Similarly, cardiotoxicity is a frequent occurrence in patients undergoing cancer chemotherapy. However, the currently available biomarkers for these common types of drug-induced toxicities have limited sensitivity or predictive value. The proteomic tools available today are enabling us to tap into the wealth of genome sequence information to discover and carefully investigate associations of thousands of proteins with drug-induced toxicities. Methods for earlier, more accurate prediction and detection of toxicity can save lives by increasing the window for successful medical treatment, while identifying the best treatment methods for each patient.

### Drug-Induced Mitochondrial Toxicity

Mitochondria are recognized as the producers of the majority of energy cells need for their normal activity. Because drugs can produce toxic effects through damaging mitochondrial bioenergetics, use of the organelle can be an effective and reliable bio-sensor to predict drug safety. Classic methods used to test the toxicity of a wide range of compounds on isolated mitochondrial fractions were later replaced by novel high-throughput methods to investigate the safety of a very large number of new molecules. The assessment of "mitochondrial safety" for new discovered molecules is of interest for pharmaceutical companies, which can now select compounds lacking mitochondrial toxicity for further trials, thus avoiding the possibility of discontinuation of clinical trials later on due to mitochondrial toxicity (Pereira et al. 2009).

Many drugs used to treat these diseases can cause toxic side effects that are often due to inhibition of mitochondrial function. MitoSciences' MitoTox line of assays can identify drug toxicity before symptoms start to appear. Tests under development

will enable physicians to identify adverse effects from drugs used to treat HIV/AIDS, hepatitis, and other infectious diseases. Several assays for detection of molecular toxicology are commercially available.

### Gene Expression Studies

Gene expression is used widely to assess the response of cells to various substances. Two technologies will be described to illustrate the use in molecular toxicology studies.

*DNA Microarrays* These allow the monitoring of the expression levels of thousands of genes simultaneously and can be used as a highly sensitive and informative method for toxicogenomics. Transcript profiling technology has been used to predict adverse toxicity for novel or untested compounds. cDNA microarray platforms have been designed specifically for gene expression events of relevance to a large number of toxicological endpoints. Such arrays allow comprehensive coverage of genes associated with entire pathways (such as oxidative stress, signal transduction, stress response, epithelial biology) and enable simultaneous measurement of more several thousand gene expression events.

*Ames Mutagenicity Assays (Xenometrix)* The Ames MPF and Ames II assays are modified versions of the Ames test using liquid culture instead of agar plates. Advantages of this format are the lower amount of sample needed and much easier handling.

Cytotoxicity assays were among the first in vitro bioassay methods used to predict toxicity of drugs to various tissues. Xenometrix offers a broad range of cytotoxicity assays for the in vitro evaluation of cells in response to pharmaceutical or chemical compounds. They are based on well established, sensitive and reliable endpoints of cytotoxicity and growth inhibition and are adapted for high throughput in microtiter plates.

## *Pharmacogenetics in Clinical Trials*

Currently, the most significant polymorphisms in causing genetic differences in phase I drug metabolism are known and therapeutic failures or adverse drug reactions caused by polymorphic genes can be predicted for several drugs. Further investigations need to be done on the consequences of each pharmacogenetic phenomenon. Pharmacokinetic or pharmacodynamic changes my determine drug selection or dose adjustment. This information can be used by the pharmaceutical industry for drug development.

Patients are being genotyped in clinical trials. Application of benefit of this approach in needs to be verified in prospective clinical trials using the parameters of

reduction in adverse drug reactions, improved outcome and cost-effectiveness. There are two approaches to application of pharmacogenetics for determining drug response profiles: candidate gene approach and SNP profile approach.

*Candidate Gene Approach* This approach involves generation of specific hypotheses about genes that cause variations in drug responses, which are then tested in responders and non-responders. Candidate drugs that are selectively metabolized by polymorphic enzymes can be dropped early in drug screening. Thus there will be fewer dropouts from late-stage clinical trials. Based on the results of clinical trials, pharmacogenetic genotyping can be introduced into routine clinical practice.

*SNP Profile Approach* This involves search for SNP profiles that correspond to efficacy or adverse events in suitable populations. It will be possible, over the next few years, to use advances in SNP mapping technology to correlate information from patients' DNA with their response to medicines. This provides significant opportunities to enhance current drug surveillance systems by collecting data that would enable rare serious adverse events to be predicted in subsequent patients before the medicine is prescribed.

An important challenge in defining pharmacogenetic traits is the need for well-characterized patients who have been uniformly treated and systematically evaluated to make it possible to quantitate drug response objectively. Therefore, it should be the routine to obtain genomic DNA from all patients enrolled in clinical drug trials, along with appropriate consent to permit pharmacogenetic studies. Because of marked population heterogeneity, a specific genotype may be important in determining the effects of a medication for one population or disease but not for another; therefore, pharmacogenomic relations must be validated for each therapeutic indication and in different racial and ethnic groups.

## *Postmarketing Pharmacogenetics*

An example of application of pharmacogenetics in post-marketing phase of Abacavir (GlaxoSmithKline), which is commonly applied in a triple therapy against HIV. Between 3 % and 5 % of the patients are hypersensitive to abacavir and have risk of various reactions including anaphylactic shock. The company is aiming to design a test, which would help the physicians to decide which patients can receive it safely. A retrospective case-control study is being conducted in two phases all subjects identified from GlaxoSmithKline studies. The first phase includes a study of candidate genes: 114 markers including HLA-A, -B and -DR. The second phase includes whole genome SNP analysis. The goal of the study is 100 cases and 200 controls matched in 1:2 ratio. This will enable detection of a difference in frequency of 15–20 % with 80 % power. Results from various projects of GlaxoSmithKline suggest that association studies can be applied successfully to pharmacogenetics and the company is committed to the use of SNPs throughout the drug discovery process.

## Clinical Implications of Pharmacogenetics

### *Application of CYP450 Genotyping in Clinical Practice*

The polymorphic nature of the CYP450 genes, which greatly affects individual drug response and adverse reactions, includes CNVs, missense mutations, insertions and deletions, and mutations affecting gene expression and activity of mainly CYP2A6, CYP2B6, CYP2C9, CYP2C19 and CYP2D6, which have been extensively studied and well characterized. CYP1A2 and CYP3A4 expression varies significantly, and the cause has been suggested to be mainly of genetic origin but the exact molecular basis remains unknown. This variability is of greatest importance for treatment with several antidepressants, antipsychotics, antiulcer drugs, anti-HIV drugs, anticoagulants, antidiabetics and the anticancer drug tamoxifen. Pharmacoepigenetics shows how gene methylation influences the expression of CYP. In addition microRNA (miRNA) regulation of P450 has been described. A review has concluded that the pharmacogenetic knowledge regarding CYP polymorphism now developed to a stage where it can be implemented in drug development and in clinical routine for specific drug treatments, thereby improving the drug response and reducing costs for drug treatment (Ingelman-Sundberg et al. 2007).

### *Pharmacogenomic Biomarker Information in Drug Labels*

A review of 1,200 drug labels of FDA-approved drugs in the US from 1945 to 2005 revealed that 121 contained pharmacogenomic information: 69 referred to human genomic biomarkers, and 52 referred to microbial genomic biomarkers. Of the labels referring to human biomarkers, 43 (62 %) pertained to polymorphisms in cytochrome P450 enzyme metabolism, with CYP2D6 being most common. Of 36.1 million patients whose prescriptions were processed by a large pharmacy benefits manager in 2006, about 8.8 million, i.e., approximately one fourth, received one or more drugs with human genomic biomarker information in the drug label (Frueh et al. 2008). The study concluded that incorporation and appropriate use of pharmacogenomic information in drug labels should be tested for its ability to improve drug use and safety in the US. Currently, there are labels for >141 FDA-approved drugs that contain proper pharmacogenomic biomarker information (see Table 4.9). There is a need for increasing this number.

### *Genotype-Based Drug Dose Adjustment*

Genotype-based drug dose adjustment information can be useful when the drug is introduced into clinical practice and would enable the dose adjustment for individualized therapy. Genetically determined interpatient variability or variations in

**Table 4.9** Pharmacogenomic biomarkers in drug labeling

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Abacavir | Infectious diseases | HLA-B | HLA-B*5701 allele carriers |
| Ado-trastuzumab emtansine | Oncology | ERBB2 | HER2 protein overexpression or gene amplification positive |
| Afatinib | Oncology | EGFR | EGFR exon 19 deletion or exon 21 substitution (L858R) mutation positive |
| Amitriptyline | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Anastrozole | Oncology | ESR1, PGR | Hormone receptor positive |
| Aripiprazole | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Arsenic trioxide | Oncology | PML/RARA | PML/RARα (t(15;17)) gene expression positive |
| Atomoxetine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Atorvastatin | Endocrinology | LDLR | Homozygous familial hypercholesterolemia |
| Azathioprine | Rheumatology | TPMT | TPMT intermediate or poor metabolizers |
| Belimumab | Autoimmune diseases | BAFF/TNFSF13B | CD257 positive |
| Boceprevir | Infectious diseases | IFNL3 | IL28B rs12979860 T allele carriers |
| Bosutinib | Oncology | BCR/ABL1 | Philadelphia chromosome (t(9;22)) positive |
| Brentuximab Vedotin | Oncology | TNFRSF8 | CD30 positive |
| Busulfan | Oncology | Ph Chromosome | Ph Chromosome negative (lack of effectiveness with Ph positive) |
| Capecitabine | Oncology | DPYD | DPD deficient |
| Carbamazepine (1) | Neurology | HLA-B | HLA-B*1,502 allele carriers |
| Carbamazepine (2) | Neurology | HLA-A | HLA-A*3,101 allele carriers |
| Carglumic Acid | Metabolic disorders | NAGS | N-acetylglutamate synthase deficiency |
| Carisoprodol | Rheumatology | CYP2C19 | CYP2C19 poor metabolizers |
| Carvedilol | Cardiology | CYP2D6 | CYP2D6 poor metabolizers |
| Celecoxib | Rheumatology | CYP2C9 | CYP2C9 poor metabolizers |
| Cetuximab (1) | Oncology | EGFR | EGFR protein expression positive |
| Cetuximab (2) | Oncology | KRAS | KRAS codon 12 and 13 mutation negative |
| Cevimeline | Dermatology | CYP2D6 | CYP2D6 poor metabolizers |
| Chloroquine | Infectious diseases | G6PD | G6PD deficient |

(continued)

**Table 4.9** (continued)

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Chlorpropamide | Endocrinology | G6PD | G6PD deficient |
| Cisplatin | Oncology | TPMT | TPMT intermediate or poor metabolizers |
| Citalopram (1) | Psychiatry | CYP2C19 | CYP2C19 poor metabolizers |
| Citalopram (2) | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Clobazam | Neurology | CYP2C19 | CYP2C19 poor metabolizers |
| Clomipramine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Clopidogrel | Cardiology | CYP2C19 | CYP2C19 intermediate or poor metabolizers |
| Clozapine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Codeine | Anesthesiology | CYP2D6 | CYP2D6 poor metabolizers |
| Crizotinib | Oncology | ALK | ALK gene rearrangement positive |
| Dabrafenib (1) | Oncology | BRAF | BRAF V600E mutation positive |
| Dabrafenib (2) | Oncology | G6PD | G6PD deficient |
| Dapsone | Dermatology | G6PD | G6PD deficient |
| Dasatinib | Oncology | BCR/ABL1 | Philadelphia chromosome (t(9;22)) positive; T315I mutation-positive |
| Denileukin diftitox | Oncology | IL2RA | CD25 antigen positive |
| Desipramine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Dexlansoprazole (1) | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizers |
| Dexlansoprazole (2) | Gastroenterology | CYP1A2 | CYP1A2 genotypes |
| Dextromethorphan and Quinidine | Neurology | CYP2D6 | CYP2D6 poor metabolizers |
| Diazepam | Psychiatry | CYP2C19 | CYP2C19 poor metabolizers |
| Doxepin | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Drospirenone and ethinyl estradiol | Neurology | CYP2D6 | CYP2D6 poor metabolizers |
| Eltrombopag (1) | Hematology | F5 | Factor V Leiden carriers |
| Eltrombopag (2) | Hematology | SERPINC1 | Antithrombin III deficient |
| Erlotinib (1) | Oncology | EGFR | EGFR protein expression positive |
| Erlotinib (2) | Oncology | EGFR | EGFR exon 19 deletion or exon 21 substitution (L858R) positive |
| Esomeprazole | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizers |
| Everolimus (1) | Oncology | ERBB2 | HER2 protein overexpression positive |
| Everolimus (2) | Oncology | ESR1 | Estrogen receptor positive |
| Exemestane | Oncology | ESR1 | Estrogen receptor positive |

(continued)

**Table 4.9** (continued)

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Fluorouracil (1) | Dermatology | DPYD | DPD deficient |
| Fluorouracil (2) | Oncology | DPYD | DPD deficient |
| Fluoxetine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Flurbiprofen | Rheumatology | CYP2C9 | CYP2C9 poor metabolizers |
| Fluvoxamine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Fulvestrant | Oncology | ESR1 | Estrogen receptor positive |
| Galantamine | Neurology | CYP2D6 | CYP2D6 poor metabolizers |
| Gefitinib | Oncology | EGFR | EGFR protein expression positive |
| Glimepiride | Endocrinology | G6PD | G6PD deficient |
| Glipizide | Endocrinology | G6PD | G6PD deficient |
| Glyburide | Endocrinology | G6PD | G6PD deficient |
| Ibritumomab tiuxetan | Oncology | MS4A1 | CD20 positive |
| Iloperidone | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Imatinib (1) | Oncology | KIT | c-KIT D816V mutation negative |
| Imatinib (2) | Oncology | BCR/ABL1 | Philadelphia chromosome (t(9;22)) positive |
| Imatinib (3) | Oncology | PDGFRB | PDGFR gene rearrangement positive |
| Imatinib (4) | Oncology | FIP1L1/PDGFRA | FIP1L1/PDGFRα fusion kinase (or CHIC2 deletion) positive |
| Imipramine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Indacaterol | Pulmonary | UGT1A1 | UGT1A1 *28 allele homozygotes |
| Irinotecan | Oncology | UGT1A1 | UGT1A1*28 allele carriers |
| Isosorbide and hydralazine | Cardiology | NAT1-2 | Slow acetylators |
| Ivacaftor | Pulmonary | CFTR | CFTR G551D carriers |
| Lansoprazole | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizer |
| Lapatinib | Oncology | ERBB2 | HER2 protein |
| Lenalidomide | Hematology | del (5q) | Chromosome 5q deletion |
| Letrozole | Oncology | ESR1, PGR | Hormone receptor positive |
| Lomitapide | Endocrinology | LDLR | Homozygous familial hypercholesterolemia and LDL receptor mutation deficient |
| Mafenide | Infectious diseases | G6PD | G6PD deficient |
| Maraviroc | Infectious diseases | CCR5 | CCR5 positive |
| Mercaptopurine | Oncology | TPMT | TPMT intermediate or poor metabolizers |
| Methylene Blue | Hematology | G6PD | G6PD deficient |
| Metoclopramide | Gastroentrology | CYB5R1-4 | NADH cytochrome b5 reductase deficient |
| Metoprolol | Cardiology | CYP2D6 | CYP2D6 poor metabolizers |

(continued)

**Table 4.9** (continued)

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Mipomersen | Endocrinology | LDLR | Homozygous familial hypercholesterolemia and LDL receptor mutation deficient |
| Modafinil | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Mycophenolic Acid | Transplantation | HPRT1 | HGPRT deficient |
| Nalidixic Acid | Infectious diseases | G6PD | G6PD deficient |
| Nefazodone | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Nilotinib (1) | Oncology | BCR/ABL1 | Philadelphia chromosome (t(9 :22)) positive |
| Nilotinib (2) | Oncology | UGT1A1 | UGT1A1*28 allele homozygotes |
| Nitrofurantoin | Infectious diseases | G6PD | G6PD deficient |
| Nortriptyline | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Ofatumumab | Oncology | MS4A1 | CD20 positive |
| Omacetaxine | Oncology | BCR/ABL1 | BCR-ABL T315I |
| Omeprazole | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizers |
| Panitumumab (1) | Oncology | EGFR | EGFR protein expression positive |
| Panitumumab (2) | Oncology | KRAS | KRAS codon 12 and 13 mutation negative |
| Pantoprazole | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizers |
| Paroxetine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| PEG-3,350, $Na_2So_4$, NaCl, KCl, sodium ascorbate | Gastroenterology | G6PD | G6PD deficient |
| Peginterferon alfa-2b | Infectious diseases | IFNL3 | IL28B rs12979860 T allele carriers |
| Pegloticase | Rheumatology | G6PD | G6PD deficient |
| Perphenazine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Pertuzumab | Oncology | ERBB2 | HER2 protein overexpression positive |
| Phenytoin | Neurology | HLA-B | HLA-B*1,502 allele carriers |
| Pimozide | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Ponatinib | Oncology | BCR/ABL1 | Philadelphia chromosome (t(9;22)) positive, BCR –ABL T315I mutation |
| Prasugrel | Cardiology | CYP2C19 | CYP2C19 poor metabolizers |
| Pravastatin | Endocrinology | LDLR | Homozygous familial hypercholesterolemia and LDL receptor deficient |
| Primaquine | Infectious diseases | G6PD | G6PD deficient |

(continued)

**Table 4.9** (continued)

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Propafenone | Cardiology | CYP2D6 | CYP2D6 poor metabolizers |
| Propranolol | Cardiology | CYP2D6 | CYP2D6 poor metabolizers |
| Protriptyline | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Quinidine | Cardiology | CYP2D6 | CYP2D6 poor metabolizers |
| Quinine Sulfate | Infectious diseases | G6PD | G6PD deficient |
| Rabeprazole | Gastroenterology | CYP2C19 | CYP2C19 poor metabolizers |
| Rasburicase | Oncology | G6PD | G6PD deficient |
| Regorafenib (1) | Oncology | VEGF | VEGFR positive |
| Regorafenib (2) | Oncology | KRAS | KRAS wt |
| Regorafenib (3) | Oncology | EGFR | EGFR positive |
| Rifampin, isoniazid, and pyrazinamide | Infectious diseases | NAT1-2 | Slow inactivators |
| Risperidone | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Rituximab | Oncology | MS4A1 | CD20 positive |
| Rosuvastatin | Endocrinology | LDLR | Homozygous and Heterozygous familial hypercholesterolemia |
| Sodium nitrite | Antidotal therapy | G6PD | G6PD deficient |
| Succimer | Hematology | G6PD | G6PD deficient |
| Sulfamethoxazole and trimethoprim | Infectious diseases | G6PD | G6PD deficient |
| Tamoxifen (1) | Oncology | ESR1, PGR | Hormone receptor positive |
| Tamoxifen (2) | Oncology | F5 | Factor V Leiden carriers |
| Tamoxifen (3) | Oncology | F2 | Prothrombin mutation G20210A |
| Telaprevir | Infectious diseases | IFNL3 | IL28B rs12979860 T allele carriers |
| Terbinafine | Infectious diseases | CYP2D6 | CYP2D6 poor metabolizers |
| Tetrabenazine | Neurology | CYP2D6 | CYP2D6 poor metabolizers |
| Thioguanine | Oncology | TPMT | TPMT poor metabolizer |
| Thioridazine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Ticagrelor | Cardiology | CYP2C19 | CYP2C19 poor metabolizers |
| Tolterodine | Urology | CYP2D6 | CYP2D6 poor metabolizers |
| Tositumomab | Oncology | MS4A1 | CD20 antigen positive |
| Tramadol and Acetaminophen | Rheumatology | CYP2D6 | CYP2D6 poor metabolizers |
| Trametinib | Oncology | BRAF | BRAF V600E/K mutation positive |
| Trastuzumab | Oncology | ERBB2 | HER2 protein overexpression positive |
| Tretinoin | Oncology | PML/RARA | PML/RARα (t(15;17)) gene expression positive |
| Trimipramine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Valproic Acid (1) | Neurology | POLG | POLG mutation positive |

(continued)

**Table 4.9** (continued)

| Drug | Therapeutic area | Gene | Referenced subgroup |
|---|---|---|---|
| Valproic Acid (2) | Neurology | NAGS, CPS1, ASS1, OTC, ASL, ABL2 | Urea cycle enzyme deficient |
| Velaglucerase Alfa | Metabolic disorders | GBA | Lysosomal glucocerebrosidase enzyme |
| Vemurafenib | Oncology | BRAF | BRAF V600E mutation positive |
| Venlafaxine | Psychiatry | CYP2D6 | CYP2D6 poor metabolizers |
| Voriconazole | Infectious diseases | CYP2C19 | CYP219 intermediate or poor metabolizers |
| Warfarin (1) | Cardiology or Hematology | CYP2C9 | CYP2C9 intermediate or poor metabolizers |
| Warfarin (2) | Cardiology or Hematology | VKORC1 | VKORC1 rs9923231 A allele carriers |



expression in some of the polymorphic enzymes are of interest to the practicing physicians. The clinical significance of genetic polymorphisms and other genetic factors may be related to substrate, metabolite, or the major elimination pathway. Genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism.

- Efficient metabolism (EM) is characteristic of normal population.
- Poor metabolism (PM) is associated with accumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation or deletion of both alleles for phenotypic expression.
- Ultrarapid metabolism (UM) results in increased drug metabolism and is an autosomal dominant trait arising from gene amplification.

Many selective serotonin reuptake inhibitors interact with CYP2D6 enzyme. The most notable example of this is fluoxetine. Through competition with CYP2D6 substrates, these drugs precipitate a drug-induced poor metabolism phenotype. It is likely that effects of CYP2D6 inhibitors on the metabolism of CYP2D6 substrates would be more pronounced in heterozygous extensive metabolism. This, however, has not been proven as yet. Clinical significance of CYP2C19 polymorphism has not yet been fully investigated as yet. Considering the relative abundance of this enzyme and the significant number of pharmaceutical substrates, clinical significance is likely to be significant.

## Use of Pharmacogenetics in Clinical Pharmacology

### *Application of CYP2C19 Pharmacogenetics for Personalized Medicine*

CYP2C19 gene has ~2,000 reference SNPs containing 28 registered alleles in the P450 Allele Nomenclature Committee and the number continues to increase. However, biological functions of CYP2C19 SNPs is not fully understood. Functional information on the variant is essential for justifying its clinical use. Mostly only common variants (minor allele frequency >5 %) that represent CYP2C19*2, *3, *17, and others have been studied. Discovery of new genetic variants is outstripping the generation of knowledge on the biological meanings of existing variants. Alternative strategies may be needed to fill this gap. A study has summarized up-to-date knowledge on functional CYP2C19 variants discovered in phenotyped humans studied at the molecular level in vitro (Lee et al. 2013). Understanding the functional significance of CYP2C19 variants is an essential step toward shifting the current medical paradigm to highly personalized therapeutic regimens.

### *Genotyping for Identifying Responders to Sulfasalazine*

One example of importance of pharmacogenetics in determining drug efficacy is that of sulfasalazine – an effective agent for chronic discoid lupus erythematosus (CDLE) – where the response to treatment is varies considerably between patients and is also unpredictable. The reason for this might relate to differences in metabolism of the drug, which is extensively acetylated by the polymorphic enzyme N-acetyltransferase 2 (NAT2). Genetic polymorphism of NAT2 is responsible for differences in the response to sulfasalazine in patients with CDLE. Genotyping can predict outcome of treatment according to whether the patients are slow acetylators (SAs) or rapid acetylators (RAs); non-responders are SAs whereas RAs respond to treatment with a complete or marked remission of the disease. In addition, SAs seem to be more prone to toxic events. Therefore, candidates for sulfasalazine therapy should be genotyped to identify those patients who might benefit from the drug.

### *HLA Alleles Associated with Lumiracoxib-Related Liver Injury*

Drug-induced liver injury is a rare but serious side effect seen in a subset of individuals taking certain drugs. Lumiracoxib (Novartis' Prexige), a selective cyclooxygenase-2 (COX-2) inhibitor developed for the symptomatic treatment of osteoarthritis and acute pain, was withdrawn or not approved in most major drug markets because of concerns over hepatotoxicity. A case-control genome-wide

association study on lumiracoxib-treated patients with liver injury and matched lumiracoxib-treated patients without liver injury (controls) identifies risk SNPs in the major histocompatibility complex (MHC) class II region in patients with lumiracoxib-related liver injury (Singer et al. 2010). Fine mapping identified a strong association to a common HLA haplotype (HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102). The mapping of the genetic association to the MHC class II region suggests a possible immunological mechanism for lumiracoxib-related hepatotoxicity as previous studies have also linked MHC genes to other adverse drug effects such as hypersensitivity to the HIV drug abacavir and liver injury associated with the antibiotic flucloxacillin. These results offer the potential to improve the safety profile of lumiracoxib by identifying individuals at elevated risk for liver injury and excluding them from lumiracoxib treatment.

## *Pharmacogenetic Basis of Thiopurine Toxicity*

Thiopurine S-methyltransferase (TPMT) catalyzes the S-methylation of thiopurine drugs. TPMT genetic polymorphisms represent a striking example of the potential clinical value of pharmacogenetics. Subjects homozygous for TPMT*3A (an allele that encodes a protein with two changes in amino acid sequence), which is the most common variant allele for low activity, are at greatly increased risk for life-threatening toxicity when treated with standard doses of thiopurines. These subjects have virtually undetectable levels of TPMT protein. TPMT*3A results in protein misfolding and aggregation in vitro. Results of studies on this topic provide an insight into a unique pharmacogenetic mechanism by which common polymorphisms affect TPMT protein function and, as a result, alter therapeutic response to thiopurine drugs.

TPMT testing prior to the prescription of azathioprine in autoimmune diseases is one of the few examples of a pharmacogenetic test that has made the transition from research into clinical practice. TPMT testing could lead to improved prescribing of azathioprine resulting in a reduction in ADRs as well as an improvement in effectiveness. A prospective economic evaluation was conducted in the UK alongside the TARGET (TPMT: Azathioprine Response to Genotype and Enzyme Testing) study, a pragmatic controlled trial that randomized (1:1) patients to undergo TPMT genotyping before azathioprine or current practice (Thompson et al. 2014). The study concluded that TPMT genotyping potentially offers a less expensive alternative than current practice, but it may also have a small but negative effect on health status. These findings are associated with significant uncertainty, and the causal effect of TPMT genotyping on changes in health status and health care resource use remains uncertain. The results from this study therefore pose a difficult challenge to decision makers.

## *Tranilast-Induced Hyperbilirubinemia due to Gene Polymorphism*

PRESTO (Prevention of REStenosis with Tranilast) was a double-blind placebo-controlled trial of Tranilast (GlaxoSmithKline) for the treatment of restenosis after percutaneous transluminal coronary angioplasty. Tranilast inhibits the release or production of cyclooxygenase-2 and restores cytokine-induced nitric oxide production. Hyperbilirubinemia developed in 4 % of the patients. Pharmacogenetic studies showed that it to be Gibert's syndrome due to polymorphism in the uridine diphosphat glucuronosyltransferase 1A1 gene – mild chronic hyperbilirubinemia that can occur in the absence of liver disease and hemolysis and is not life-threatening. The trials continued although the final results were lack of efficacy.

## *Linking Pharmacogenetics with Pharmacovigilance*

### Genetic Susceptibility to ADRs

A simple method has been devised that is acceptable to members of the general population to enable estimation of the risks that specific genetic factors confer on susceptibility to specific ADRs. Buccal swabs are a minimally invasive method to obtain cells for DNA extraction. A pilot study using this approach was conducted in the New Zealand Intensive Medicines Monitoring Program to link prescription event monitoring (PEM) studies with pharmacogenetics. Use of buccal swabs was acceptable to patients and provided DNA of sufficient quantity and quality for genotyping. Although no differences in the distribution of genotypes in the case and control populations were found in this small study, case-control studies investigating genetic risks for adverse drug reactions using drug cohorts from PEM studies are possible, and there are several areas where population-based studies of genetic risk factors for are needed:

- Genetic variations affecting P-gp function
- Variations affecting drugs metabolized by CYP2C9 and other polymorphic CYP enzymes
- Genetic variation in β-adrenergic receptors and adverse outcomes from β-adrenoceptor agonist therapy
- Genetic variation in cardiac cell membrane potassium channels and their association with long QT syndromes and serious cardiac dysrhythmias.

### Linking Genetic Testing to Postmarketing ADR Surveillance

FDA is interested in collaboration with consumer personal genomics companies for tracking post-marketing ADR surveillance. In marketing ancestry and disease-predisposition genetic testing services directly to consumers, personal genomics

companies are building large electronic databases of clinical and genomic information that the FDA believes can be useful in tracking ADRs in a post-marketing setting. It may be possible to investigate if customers with certain genetic polymorphisms are on certain drugs and have experienced certain ADRs. As a part of FDA Amendment Act, which was signed into law in 2008, pharmaceutical companies are required to submit results from post-marketing studies to a clinical trial registry. By partnering with personal genomics companies, the FDA would gain access to genomic data that may provide additional insight into ADRs that have genetic underpinnings. Such a collaborative project would probably not be possible until companies were at the point where they had genotyped at least 100,000 patients on high-density arrays. One current potential drawback to an alliance between the FDA and personal genomics firms is that, at the moment, the cost for such services is out of reach for the average consumer, which could limit the diversity of individuals contained in a database.

### *Recommendations for the Clinical Use of Pharmacogenetics*

Due to the rapid development of cost-effective methods for genotyping and the need to genotype only once in the lifetime of a patient, it would be advisable to include the genotype in the patient's record. It is also desirable to include the genotypes of transport proteins and drug receptors, which can reveal highly predictive genetic information. This would provide the physician with valuable information to individualize the treatment. Besides development of personalized medicines, the impact of genotyping on medical practice would shift the emphasis from present diagnosis-based treatment to detection of disease prior to clinical manifestation and preventive treatment with appropriate medicine and a dose that is most effective and safest for an individual.

Predicted clinical developments from application of pharmacogenetics are:

- Establishment of prescribing guidelines, based on clinical studies, for drugs that are subject to substantial polymorphic metabolism
- Prescribing advice will relate dose to genotype and will highlight the possibility of drug interactions when multiple drugs are prescribed concomitantly
- Establishment and recording of individual patient genotypes that is, "personal pharmacogenetic profiles"
- Pharmacogenetic testing will substantially reduce the need for hospitalization, and its associated costs, because of adverse drug reactions
- Development of new drugs for patients with specific genotypes that is, "drug stratification"

## Limitations of Pharmacogenetics

Inherited component of the response to drugs is often polygenic. Furthermore, the drug response is probably affected by multiple genes, each gene with multiple polymorphisms distributed in the general population. Racial differences add further

confounding factors. Drug response might be predicted from a certain pattern of polymorphisms rather than only a single polymorphism, yet these patterns probably differ between ethnic groups. This could prevent predictions about drug responses across the general patient population, and it emphasizes the need to stratify clinical pharmacogenomics studies.

SNP maps and candidate-gene strategies are based on existing knowledge of a medication's mechanisms of action and pathways of metabolism and disposition. The candidate-gene strategy has the advantage of focusing resources on a manageable number of genes and polymorphisms that are likely to be important but the limitations are the incompleteness of knowledge of a medication's pharmacokinetics and mechanisms of action.

The dynamic complexity of the human genome, involvement of multiple genes in drug responses, and racial differences in the prevalence of gene variants impede effective genome-wide scanning and progress towards practical clinical applications. Genomic technologies are still evolving rapidly, at an exponential pace similar to the development of computer technology over the past 20 years. Gene expression profiling and proteomic studies are evolving strategies for identifying genes that may influence drug response.

Ethical issues also need to be resolved. Holding sensitive information on someone's genetic make-up raises questions of privacy and security and ethical dilemmas in disease prognosis and treatment choices. After all, polymorphisms relevant to drug response may overlap with disease susceptibility, and divulging such information could jeopardize an individual. On the other hand, legal issues may force the inclusion of pharmacogenomics into clinical practice. Once the genetic component of a severe adverse drug effect is documented, doctors may be obliged to order the genetic test to avoid malpractice litigation.

## Pharmacoepigenomics vs Pharmacogenetics in Drug Safety

Phamacoepigenomics refers to drug action as influenced by the epigenome, which is the overall epigenetic state of a cell, and serves as an interface between the environment and the genome. The role of epigenetic factors in drug action has been mentioned throughout this report. The epigenome is dynamic and responsive to environmental signals not only during development, but also throughout life; and it is becoming increasingly apparent that chemicals can cause changes in gene expression that persist long after exposure has ceased. A hypothesis has been presented, which states that commonly-used pharmaceutical drugs can cause such persistent epigenetic changes (Csoka and Szyf 2009). Drugs may alter epigenetic homeostasis by direct or indirect mechanisms. Direct effects may be caused by drugs which affect chromatin architecture or DNA methylation. For example the antihypertensive hydralazine inhibits DNA methylation. An example of an indirectly acting drug is isotretinoin, which has transcription factor activity. A two-tier mechanism is

postulated for indirect effects in which acute exposure to a drug influences signaling pathways that may lead to an alteration of transcription factor activity at gene promoters. This stimulation results in the altered expression of receptors, signaling molecules, and other proteins necessary to alter genetic regulatory circuits. With more chronic exposure, cells adapt by an unknown hypothetical process that results in more permanent modifications to DNA methylation and chromatin structure, leading to enduring alteration of a given epigenetic network. Therefore, any epigenetic side-effect caused by a drug may persist after the drug is discontinued. It is further proposed that some iatrogenic diseases such as tardive dyskinesia and drug-induced systemic lupus erythematosus are epigenetic in nature. If this hypothesis is correct the consequences for modern medicine are profound, since it would imply that our current understanding of pharmacology is an oversimplification. Thus epigenetic side-effects of pharmaceuticals may be involved in the etiology of heart disease, cancer, neurological and cognitive disorders, obesity, diabetes, infertility, and sexual dysfunction. It is suggested that a systems biology approach employing microarray analyses of gene expression and methylation patterns can lead to a better understanding of long-term side-effects of drugs, and that in the future, epigenetic assays should be incorporated into the safety assessment of all pharmaceutical drugs. The impact of pharmacoepigenomics may be equal to or greater than that of pharmacogenetics.

## Future Role of Pharmacogenetics in Personalized Medicine

The number of polymorphisms identified in genes encoding drug metabolizing enzymes, drug transporters, and receptors is rapidly increasing. In many cases, these genetic factors have a major impact on the pharmacokinetics and pharmacodynamics of a particular drug and thereby influence the sensitivity to such drug in an individual patient with a certain genotype. The highest impact is seen for drugs with a narrow therapeutic index, with important examples emerging from treatment with antidepressants, oral anticoagulants, and cytostatics, which are metabolized by CYP4502D6, CYP2C9, and TPMT, respectively. Many of the genes examined in early studies were linked to highly penetrant, single-gene traits, but future advances hinge on the more difficult challenge of elucidating multi-gene determinants of drug response.

In order to apply the increasing amount of pharmacogenetic knowledge to clinical practice, specific dosage recommendations based on genotypes will have to be developed to guide the clinician, and these recommendations will have to be evaluated in prospective clinical studies. Such development will lead to personalized medicines, which hopefully would be more efficient and will result in fewer adverse drug reactions.

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# Chapter 5
# Pharmacogenomics

## Introduction

The total genetic material of an organism, that is, an organism's complete deoxyribonucleic acid (DNA) sequence is called a genome and genomics is the study of all of the genes in an organism – their sequences, structure, regulation, interaction, and products. Currently, it is estimated that there are approximately 19,000 genes in the human organism. Several new technologies have been developed to study the genome and new terms have been derived from genomics, the best known of which is pharmacogenomics. Completion of sequencing of the human genome has opened a new era for improved understanding of the genetic basis of human diseases and to provide new targets for drug discovery. Pharmacogenomics is an important basis for the development of personalized medicines.

Pharmacogenomics implies the use of genetic sequence and genomics information in patient management to enable therapy decisions. The genetic sequence and genomics information can be that of the host (normal or diseased) or of the pathogen. Pharmacogenomics will have an impact on all phases of drug development – from drug discovery to clinical trials. It will also apply to a wide range of therapeutic products including bioengineered proteins, cell therapy, antisense therapy and gene therapy. These treatments are also subject to constraints and complexities engendered by individual variability. Role of pharmacogenomics in variable therapy targets is shown in Table 5.1.

## Basics of Pharmacogenomics

Pharmacogenomics applies the large-scale systemic approaches of genomics to drug discovery and development. It also involves the study of the mechanisms by which drugs change the expression of genes, including drug-metabolizing enzymes, a phenomenon known as induction. Various technologies enable the analysis of

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**Table 5.1** Role of pharmacogenomics in variable therapy targets

| Variable target | Therapy/prevention | Disease |
|---|---|---|
| AlloMap® gene profile | Immunosuppressive drugs | Heart transplant rejection |
| Alpha-adducin | ACE inhibitors | Hypertension |
| BCR-abl; c-KIT | Gleevec/Imatinib | Cancer/CML |
| BRCA1/2 | Surveillance; tamoxifen; prophylactic surgery | Breast and ovarian cancer |
| CETP | HMG-CoA reductase inhibitors | Atherosclerosis |
| CYP2C9/VKORC1 | Warfarin | Coagulation disorders |
| CYP2D6/2D19 (Amplichip®) | ~25 % of prescribed drugs | Drug metabolism in disease |
| EGFR | Tarceva, Iressa | Lung cancer |
| Estrogen receptor | Tamoxifen | Breast cancer |
| Familion® 5-gene profile | Pharma/lifestyle prevention | Cardiac rhythm abnormalities |
| HER-2/neu receptor | Herceptin/Trastuzumab | Breast cancer |
| KRAS mutation | Tyrosine kinase inhibitors | Lung cancer drug resistance |
| MammaPrint 70-gene profile | Aduvant chemotherapy | Breast cancer recurrence |
| Oncotype DX: 16 gene profile | Chemotherapy protocols | Breast cancer recurrence |
| OncoVue® (117 loci) | Surveillance | Sporadic breast cancer |
| p16 gene/CDKN2A | Surveillance | Melanoma |
| PML-RAR alpha | Tretinoin/all trans retinoic acid | Acute myelocytic leukemia |
| Sprycel (dasatinib) | BCR-Abl | Gleevec resistance |
| TPMT | Mercaptopurine | Acute lymphocytic leukemia |
| Transcriptional profiles | Chemotherapy protocols | Non-Hodgkin's lymphoma |
| Transcriptional profiles | Chemotherapy protocols | Acute myelocytic leukemia |
| TruGene®-HIV 1 genotyping | Antiretroviral drugs | HIV virus drug resistance |
| UGT1A1 | Camptosar® (irinotecan) | Colon cancer |



these complex multifactorial situations to obtain individual genotypic and gene expression information. These same tools are applicable to study the diversity of drug effects in different populations. Pharmacogenomics promises to enable the development of safer and more effective drugs by helping to design clinical trials such that non-responders would be eliminated from the patient population and take the guesswork out of prescribing medications. It will also ensure that the right drug is given to the right person from the start. In clinical practice, doctors could test patients for specific SNPs known to be associated with non-therapeutic drug effects

before prescribing in order to determine which drug regimen best fits their genetic makeup. Pharmacogenomic studies are rapidly elucidating the inherited nature of these differences in drug disposition and effects, thereby enhancing drug discovery and providing a stronger scientific basis for optimizing drug therapy on the basis of each patient's genetic constitution.

## Pharmacogenomics vs Pharmacogenetics

Pharmacogenomics, a distinct discipline within genomics, carries on that tradition by applying the large-scale systemic approaches of genomics to understand the basic mechanisms and apply them to drug discovery and development. Pharmacogenomics now seeks to examine the way drugs act on the cells as revealed by the gene expression patterns and thus bridges the fields of medicinal chemistry and genomics. Some of the drug response markers are examples of interplay between pharmacogenomics and pharmacogenetics; both are playing an important role in the development of personalized medicines. The two terms – pharmacogenetics and pharmacogenomics – are sometimes used synonymously but one must recognize the differences between the two as shown in Table 5.2.

**Table 5.2** Pharmacogenetic vs. pharmacogenomic studies

| Feature | Pharmacogenetics | Pharmacogenomics |
|---|---|---|
| Focus of studies | Patient variability | Drug variability |
| Scope of studies | Study of sequence variations in genes suspected of affecting drug response | Studies encompass the whole genome |
| Methods of study | SNP, expression profiles and biochemistry | Gene expression profiling |
| Relation to drugs | One drug and many genomes (patients) | Many drugs and one genome |
| Examination of drug effects | Study of one drug in vivo in different patients with inherited gene variants | Examination of differential effects of several compounds on gene expression in vivo or in vitro |
| Prediction of drug efficacy | Moderate | High value |
| Prediction of drug toxicity | High value | Moderate |
| Application relevant to personalized medicine | Patient/disease-specific healthcare | Drug discovery and development or drug selection |

## Pharmacogenomics and Drug Discovery

The impact of new technologies at various stages of the drug discovery process is shown schematically in Fig. 5.1. This scheme shows that genomic technologies and pharmacogenomics play an important role in drug discovery and development. Analysis of SNP data has already led to the identification of several precandidate genes potentially useful for drug discovery. Information obtained from study of the function of genes, their interactions, their role in biological pathways, as well as their variability among the population can be utilized in drug discovery. An understanding of gene expression changes from normal tissues through the disease development process among different populations provides possible targets for drug development.

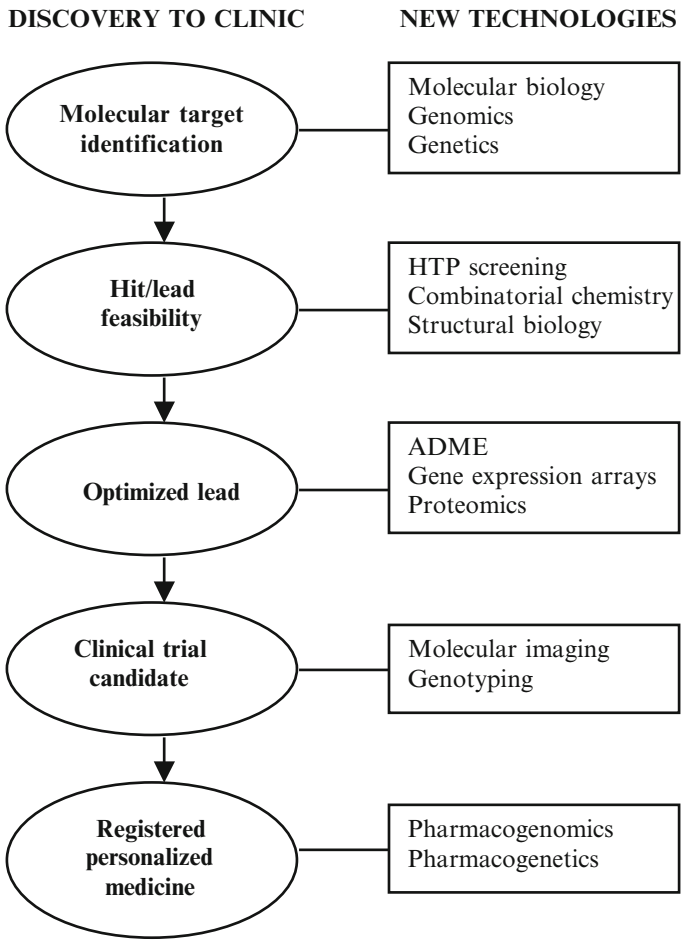


**Fig. 5.1** Impact of new technologies at various stages of the drug discovery process

Another important stage in drug discovery is lead selection that can be based equally upon biomarkers of toxicity or biomarkers of efficacy. Use of mRNA transcript profiling technology coupled to database search, enables creation of expression pharmacogenomic profiles of drug response for many classes of drugs in target tissues. These response profiles can be analyzed to uncover biomarkers that correlate with toxicity or efficacy. These biomarkers can help triage hepatotoxicity and cardiotoxicity among other response profiles and reduce the cost of drug development.

Target selection in the future should be genetics-based rather than the currently popular target validation. Use of genetic evidence-based methods of target selection should reduce the testing of too many hypotheses that are eventually proven wrong. Reducing attrition and improving a product's return on investment measure success in discovery. As molecules pass through the development pipelines, choices made in 2015 will undoubtedly play a role in the outcomes in 2020.

Most disease susceptibility genes are not drug targets by themselves. At first, knowledge of the gene has to be translated into an understanding of the role the gene-encoded protein plays in the disease. Then one has to identify a disease-related tractable target – be it an enzyme, receptor or ion channel – using the best functional genomics tools available. The difficulty of this task is indicated by the fact that almost a decade following the discovery of APOE as a disease susceptibility gene, the precise role of this gene in Alzheimer's disease has yet to be unraveled. Thus moving from a gene to an understanding of its functional role in disease, and moving from there to optimal therapeutic targets and a therapeutic agent, is the next great challenge for drug development. Genomics is expected to increase the number of possible disease targets by a factor of 5–10. This increase will be driven mainly by the genetic heterogeneity of many diseases. Thus there will be a need to develop more potential medicines that are aimed at the patients' underlying genotype, not just the disease phenotype. This increase in targets generated by genomics is being successfully met by the sophistication of technologies such as combinatorial chemistry and high-throughput screening.

## *Preclinical Prediction of Drug Efficacy*

Assays of drug action typically evaluate biochemical activity. However, accurately matching therapeutic efficacy with biochemical activity is a challenge. High-content cellular assays seek to bridge this gap by capturing broad information about the cellular physiology of drug action. Detailed information contained in genomic expression data is usually sufficient to match the physiological effect of a novel drug at the cellular level with its clinical relevance. This capacity to identify therapeutic efficacy on the basis of gene expression signatures in vitro has potential utility in drug discovery and drug target validation relevant to personalized medicine.

Knowledge of genetic variation in a target enables early assessment of the clinical significance of polymorphism through the appropriate design of preclinical studies and use of relevant animal models. A focused pharmacogenomic strategy at the preclinical phase of drug development can contribute to the decision-making process for

full development of compounds. The availability of genomic samples in large phase IV trials provides a valuable resource for further understanding the molecular basis of disease heterogeneity, providing data that feeds back into the drug discovery process in target identification and validation for the next generation of improved medicines.

## Pharmacogenomics and Clinical Trials

Examples of role of pharmacogenomics in clinical trials are listed in Table 5.3.

The knowledge of pharmacogenetics and pharmacogenomics is already improving the conduct of clinical trials based on genotyping stratification and development of individualized healthcare or personalized medicines. Current applications of pharmacogenomics include development by prospective genotyping in phase I trials, to ensure that a subject population is representative with respect to drug metabolism phenotypes. The banking of genetic material from later stage trials for retrospective studies on drug response is becoming more frequent, but is not yet standard in the industry. Retrospective studies using collections of DNA that supply medical information on specific disease types, drug response and ethnic composition could build a foundation for the evolution of medicine from diagnosis and treatment towards prediction and prognosis which are important components of integrated personalized medicine. Figure 5.2 shows various steps for the application of pharmacogenomics in clinical trials. Some examples of use of pharmacogenomics in clinical studies are shown in Table 5.4.

**Table 5.3** Role of pharmacogenomics in clinical trials

| |
|---|
| Identification of variations in large number of genes that affect drug action |
| Stratification of patients in clinical trials according to genotype |
| Reduction of the total number of patients required for clinical trials |
| Prediction of optimal doses of the drug in different patient populations |
| Reduction in drug development time by demonstrating efficacy in specific populations |
| Prediction of adverse reactions or therapeutic failures based on the genotype of the patient |
| Prediction of drug-drug interactions |



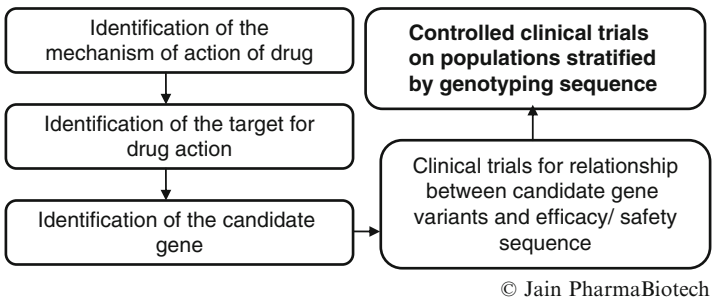


**Fig. 5.2** Steps in the application of pharmacogenomics in clinical trials

**Table 5.4** Examples of pharmacogenomics-based clinical studies

| Disease | Drug | Polymorphism | Results |
|---|---|---|---|
| Asthma | Zileutin | ALOX5 genotype | Reduced response among heterozygotes |
| Alzheimer's disease | Tacrine | ApoE4 genotype | Those with ApoE4 gene show poor response |
| Coronary heart disease | Pravastatin | Polymorphism of cholesteryl ester transfer protein at site B1B1 | Better response to pravastatin than those with polymorphism at B2B2 |
| Schizophrenia | Clozapine | 5HT2A receptor C102 allele | Improved response to clozapine |



## *Impact of Genetic Profiling on Clinical Studies*

Genotyping is important in design and interpretation of clinical studies. Advantages of molecular genetic profiling in clinical studies are:

- It is a contribution to molecular definition of the disease
- Correlation of drug response to the genetic background of the patient
- Prediction of dose-response and adverse effects
- SNP mapping data can be used to pinpoint a common set of variant nucleotides shared by people who do not respond to a drug
- Samples collected during clinical trials can be used for drug discovery

Clinical trials should be structured in such a way that all the test groups will contain adequate numbers of different phenotypes within polymorphisms. In case of a genotype-specific drug, test groups should contain only the targeted phenotypes. Molecular genetic methods may be applied both for genetic profiling (polymorphisms, mutations, etc.) of cohorts and for monitoring and guidance of therapies.

Genetic profiling can be used for stratifying subjects in clinical trials. Genotype/phenotype correlations based on identification of mutations and polymorphisms are used for population segmentation. For example, pharmaceutical companies could use the correlation data from phase I and phase II clinical trials to determine the size of the patient population that would benefit from the drug under development. They would also know the size of the clinical group needed for a phase III clinical trial to obtain statistically significant data to support the clinical development program. This number should be much lower than that required currently for phase III clinical trials because by this stage, the patients are known to have a genotype that suggests a favorable response to the drug.

Pharmacogenomic tests used by the pharmaceutical companies themselves can be used to help identify suitable subjects for clinical trials, aid in interpretation of clinical trial results, find new markets for current products and speed up the development of new treatments and therapies.

It is anticipated that genotyping at different stages of clinical trials would change the approach to drug development. Currently there are four phases of clinical trials followed by postmarketing studies. Suggestions to shorten the clinical drug development process by reducing the number of phases are as follows:

- Phase I. Genotyping and ADME studies. Selection of patients for phase II.
- Phase II. Main study.
- Phase III. May be replaced by an extension of the phase II and analysis of data to identify responders vs non-responders and those who have adverse reactions. Large-scale genotyping to discover new pharmacogenomic markers.
- Post-marketing studies. Detection of rare events and development of diagnostic tests tied in with the drug therapeutics.

Some drawbacks of pharmacogenomics-based clinical trials are:

- Exclusion of certain subjects from trials on the basis of genotype is interpreted s discrimination similar to exclusion of women and minorities
- Stratification into smaller subgroups might confound statistical analysis and interpretation of results
- Statistical differences may not be clinically significant
- Misuse of the good results in a subgroup to portray the drug as a whole
- Need to do separate clinical trials in different countries

## *Limitations of the Pharmacogenomic-Based Clinical Trials*

Large prospective trials to demonstrate the value of genotyping in patient management will be required to support the introduction of pharmacogenomics into clinical practice. Some of the limitations to be considered are:

- Such studies are costly and can be justified only if there is a reproducible association between genotype and a clinically relevant phenotype.
- Non-replication is prevalent among genetic association studies. It may reflect real population differences but multiple comparisons, biases and other design limitations suggest that many initial positive associations represent Type I errors.
- Successful detection of a true genetic effect requires not only an informed and careful selection of candidate genes but also the assiduous application of sound principles of study design.
- Independent and prospective confirmation of the hypothesized genetic effect in a population similar to the one originally studied is required.

In selected situations, pharmacogenomic studies in healthy volunteers may support a decision to perform such prospective association studies. If the results of these studies are significant and potential health or economic benefits of therapy are considerable, a major clinical trial can be considered to assess the usefulness of a pharmacogenomics-based therapy.

An alternative to prospective controlled clinical trials is simple examination of a treated population in a clinic by retrospective genotyping. This would reveal individuals that had received treatment by chance from those where it would have been recommended on basis of genotype as well as individuals that received inappropriate treatment. This approach could produce valuable data to support the value of pharmacogenomic testing.

## Current Status and Future Prospects of Pharmacogenomics

There is an ongoing process of identifying the common, biologically relevant SNPs, in particular those that are associated with the risk of disease and adverse drug reaction. The identification and characterization of these SNPs are necessary before their use as genetic tools. Most of the ongoing SNP related studies are biased deliberately towards coding regions and the data generated from them are therefore unlikely to reflect genome wide distribution of SNPs (Katara 2014). Single SNP association testing is suboptimal given the complexities of the clinical trial setting, including: (1) relatively small sample sizes; (2) diverse clinical cohorts within and across trials due to genetic ancestry (potentially impacting the ability to replicate findings); and (3) the potential polygenic nature of a drug response (Kohler et al. 2014). The authors of this proof-of-concept study propose a shift in the current paradigm to consider the gene as the genomic feature of interest in pharmacogenomics discovery. Genomic region-based association testing has the potential to improve the power of detecting single SNP or complex pharmacogenomic effects in the discovery stage and to improve power in the replication stage.

Even, the full potential of currently available data about pharmacogenomics is largely unrealized because of the logistic challenges in obtaining suitable genomic information in a timely manner to guide prescribing. Placing genomic information in the electronic medical record would facilitate personalized medicine. If the patient's entire genome were part of his or her medical record, then the complexities of acquiring a DNA sample, shipping it, and performing laboratory work would be replaced by a quick electronic query. Although this scenario holds great promise, the utility of genomic information for drug prescribing must be documented with rigorous evidence.

The first marketing authorization of a NGS platform (Illumina's MiSeqDx) for clinical use in November 2013 will expand the incorporation of genetic information to improve health care. The approval of NGS is only the beginning. There are many challenges ahead before personalized medicine can be truly embedded in health care (Collins and Hamburg 2013). There is a need to continue to uncover variants within the genome that can be used to predict disease onset, affect progression, and modulate drug response. New genomic findings need to be validated before they can be integrated into medical decision making. Doctors and other health care professionals will need support in interpreting genomic data and their meaning for individual patients. Patients will want to be able to talk about their genetic information

with their doctor. With the right information and support, patients will be able to participate alongside their doctors in making more informed decisions. Even the most promising technologies cannot fully realize their potential if the relevant policy, legal, and regulatory issues are not adequately addressed.

An important aim of pharmacogenomics is to contribute to some of the Millennium Development Goals (MDG), i.e., by providing an incentive to public research laboratories and pharmaceutical companies to develop drugs for their low income countries so that they have a fair share of advances in healthcare. A review of the literature indicates that pharmacogenomic research has focused mainly on non-communicable disease such as cancer, cardiovascular diseases, and neurological disorders but paid little attention to infections and orphan diseases (Olivier and Williams-Jones 2014). Thus research in the field of pharmacogenomics has failed in its promise to contribute to the MDGs by reducing global health inequalities.

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# Chapter 6
# Pharmacoproteomics

## Basics of Proteomics

The term 'proteomics' indicates PROTEins expressed by a genOME and is the systematic analysis of protein profiles of tissues. Proteomics parallels the related field of genomics. Now that the human genome has been sequenced, we face the greater challenge of making use of this information for improving healthcare and discovering new drugs. There is an increasing interest in proteomics technologies now because deoxyribonucleic acid (DNA) sequence information provides only a static snapshot of the various ways in which the cell might use its proteins whereas the life of the cell is a dynamic process. A detailed discussion of proteomics is given in a special report on this topic (Jain 2015). Application to development of personalized medicine will be discussed here briefly.

Role of proteomics in drug discovery and development is termed "pharmacoproteomics" and is a more functional representation of patient-to-patient variation than that provided by genotyping, which indicates its important role in the development of personalized medicine (Jain 2004). Pharmacoproteomics is parallel to pharmacogenomics and is used for subtyping patients on the basis of protein analysis. Proteomics-based characterization of multifactorial diseases may help to match a particular target-based therapy to a particular biomarker in a subgroup of patients. By classifying patients as responders and non-responders, this approach may accelerate the drug development process. Because it includes the effects of post-translational modification, pharmacoproteomics connects the genotype with the phenotype – a connection that is not always predicted by genotyping alone. For example, a silent SNP can give rise to two or more variants forms of mRNAs that do not produce an altered amino acid sequence in the proteins that are encoded, but it can alter a phenotype by inducing change in the mRNA folding. An understanding of mRNA conformational changes could lead to new drug targets such as an allele-specific target.

Proteomics-based characterization of multifactorial diseases may help to match a particular target-based therapy to a particular marker in a subgroup of patients.

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The industrial sector is taking a lead in developing this area. Individualized therapy may be based on differential protein expression rather than a genetic polymorphism.

Proteomics had a great impact on diagnosis during the first decade of the twenty-first century. By the end of the second decade protein chip-based tests will be available for several diseases. Knowledge gained from genomics and proteomics will be combined to provide optimal detection of disease at an early stage for prevention or early intervention. Proteomics-based molecular diagnostics will have an important role in the diagnosis of certain conditions and proteomics-based medicines would be integrated in the total healthcare of a patient.

Proteomics plays an important role in systems biology because most biological systems involve proteins. Proteins that are disturbed by disease and gene regulatory networks differ from their normal counterparts and these differences may be detected by multiparameter measurements of the blood. This will have a major role in creating a predictive, personalized, preventive, and participatory approach to medicine.

## Proteomic Approaches to the Study of Pathophysiology of Diseases

Most of the human diseases are multifactorial and their complexity needs to be understood at the molecular level. Genomic sequencing and mRNA-based analysis of gene expression has provided important information but purely gene-based expression data is not adequate for dissection of the disease phenotype at the molecular level. There is no strict correlation between the gene and the actual protein expression. Therefore, the cell's full proteome cannot be deciphered by analysis at the genetic level alone. It is necessary to look at the proteins directly to understand the disease at a molecular level. Aberrations in the interaction of proteins with one another are at the heart of the molecular basis of many diseases. For example, genomic analysis alone may not suffice in type 2 diabetes mellitus as the insulin gene may be normal and the disease may arise from an abnormality at any point in the complicated pathway that involves insulin and the complex proteins with which it interacts. Discovery of the mutations in BRCA1 and BRCA2 genes in familial breast cancer has not led to design of a curative therapy because function of the proteins coded by the genes is unknown. Analysis of different levels of gene expression in healthy and diseased tissues by proteomic approaches is as important as the detection of mutations and polymorphisms at the genomic level and may be of more value in designing a rational therapy.

The proteome is dynamic and reflects the conditions, such as a disease, to which a cell is exposed. Combining the genomic with the proteomics information would, therefore, reveal a more dynamic picture of the disease process. An example of the use of proteomics in understanding pathophysiology of disease is the study of

phagosome proteome. Phagosomes are required by macrophages to participate in tissue remodeling, clear dead cells, and restrict the spread of intracellular pathogens. The systematic characterization of phagosome proteins provided new insights into phagosome functions and the protein or groups of proteins involved in and regulating these functions.

An example of the utilization of the knowledge of pathomechanism of disease is the selection of key families of proteins such as G-protein coupled receptors (GPCR) and ion channel transporters that are well-established targets for intervention in disease. Maps of distribution of these proteins are available and are evaluated in the context of genomics, pharmacology and clinical information. This has led to identification of novel mechanisms for therapeutic intervention.

## Single Cell Proteomics for Personalized Medicine

Owing to the complexity of the intracellular metabolic pathways, an understanding of the intracellular pathways has been lagging behind the advances in gene expression. Recently, multicolor FACS (fluorescence activated cell sorting) techniques combined with phosophospecific antibodies have been developed, enabling the determination of relative phosphorylation of signal transduction intermediates in individual cells. When stimulated with cytokines, individual leukemia cells exhibit marked differences in phosphoprotein patterns, which correspond with disease outcome. Thus, single cell phosphoproteomic techniques are superior to other proteomic technologies for the molecular diagnosis of disease and development of personalized medicine. Although study of the phosphoprotein network is usually associated with oncology, such a technology might be useful for other diseases for which multiple treatment options exist and competing technologies have not been able to adequately predict the optimal treatment for individual patients.

## Diseases Due to Misfolding of Proteins

Taking on the right shape is vital to a protein's action. To help make sure this happens correctly, cells contain chaperone proteins devoted to helping newly made proteins fold. Other proteins, the ubiquitins, bind to proteins that have failed the shape test and mark them for destruction.

Incorrectly folded proteins are at the root of several disorders. Prion diseases are associated with misfolding of proteins and this is linked to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease. Disturbance of protein folding system leads to spinocerebellar ataxia – a fatal movement disorder of childhood. The gene mutation responsible for this disease is SCA1, which codes for a protein, ataxin1. Mutations in the gene create an enlarged portion in ataxin1 containing multiple copies of the amino acid glutamine. This stops the protein from folding

normally, causing them to clump together and form toxic deposits in neurons. The disease can also arise if neurons make too much of the normal protein, pushing the protein folding capacity of chaperones beyond their normal limits. Other genes counteract the effects of misfolded ataxin and provide potential targets for future human therapies.

In many cases, the mutations are not so severe as to render the protein biologically inactive. Rather, the mutations often result in only subtle protein-folding abnormalities. In the case of the CFTR protein, a mutation leading to the loss of a single amino acid is responsible for the diseased state in the majority of individuals with cystic fibrosis. A number of low-molecular-weight compounds, all of which are known to stabilize proteins in their native conformation, are effective in rescuing the folding and/or processing defects associated with different mutations that often lead to human disease. Recent reports have suggested that some of the major neurodegenerative pathologies could be gathered under a unifying theory stating that all diseases linked to protein misfolding could be due to the inherent toxicity associated with protein aggregates.

## *Therapies for Protein Misfolding*

A number of low-molecular-weight compounds, all of which are known to stabilize proteins in their native conformation, are effective in rescuing the folding and/or processing defects associated with different mutations that often lead to human disease. The small compounds being developed to correct the misfolding of proteins are called chemical chaperones, pharmacological chaperones or pharmacoperones. Promising results have been achieved in a small clinical trial to treat nephrogenic diabetes insipidus, and trials are under way of patients with emphysema and chronic liver disease, conditions that can be caused by the same misfolded protein. Encouraging in vitro results have been reported for cystic fibrosis, Fabry disease, hypercholesterolemia, and the aggregation of prions in spongiform encephalopathy. In mice, the mutant p53 tumor-suppressor protein has been successfully treated. Potential also exists to correct misfolding in retinitis pigmentosa, sickle cell disease, thalassemia, cataracts, and hypertrophic cardiomyopathy. This approach may offer an alternative to antibody treatments and gene therapy. Some other examples of recent achievements are as follows.

Several mutations of the GnRH (gonadotropin-releasing hormone) have been identified in patients with hypogonadotropic hypogonadism (HH) and some of the missense mutations can be rescued with a GnRH peptidomimetic antagonist that acts as folding template, stabilizing (otherwise) misfolded GnRHR receptor mutants and thereby restoring function. Antagonist can be removed after the correctly folded protein reaches the cell surface and the receptor will function normally, as measured by its participation in activating the production of inositol phosphate and release of intracellular calcium. This suggests that the drug need not interact at the same site as the native ligand; it can stabilize the protein allosterically. The pharmacoperone

acts as a scaffolding or template for folding rather than as a competitive antagonist. These findings present therapeutic opportunities for HH and other disorders resulting from protein misfolding. A synthetic antagonist has been used successfully in clinical trials to rescue receptor protein misfoldings in nephrogenic diabetes insipidus, in which improper reabsorption of water in the kidneys leads to various metabolic disorders.

The potential of chemical chaperones to treat chronic liver disease and emphysema has been established as both diseases can be caused by misfolding of the alpha-1-antitrypsin (alpha-1-AT) inhibitor. When the mutant protein is retained in the liver cells rather than secreted into the blood and body fluids, it is thought to become toxic to the liver. Its depletion in the lung causes emphysema via failure to block an enzyme that hydrolyzes the connective tissue elastin. A drug, 4-phenylbutyric acid (PBA), which was shown to be was effective on mice transgenic for the human alpha-1-AT gene, has been safely administered in clinical trials to children with disorders of the urea cycle.

## Proteomic Technologies for Drug Discovery and Development

Proteomic technologies are now being integrated into the drug discovery process as complimentary to genomic approaches. This offers the scientists the ability to integrate information from the genome, expressed mRNAs and their respective proteins as well as subcellular localization. By focusing on protein activity levels, or expression levels, researchers are able to learn more about the role proteins play in causing and treating disease. Proteomics also aids in deciphering the mechanisms of disease and increasing both the opportunity to develop drugs with reduced side effects and an increased probability of clinical trial success. Proteomics has the potential to increase substantially the number of drug targets and thereby the number of new drugs. Automation of proteomics on a scale similar to that used for genome sequencing may be needed and this is feasible by adapting the many tools already developed for genomics for application to proteomic technologies. Application of proteomic technologies has enabled the prediction of all possible protein-coding regions and to choose the best candidates among novel drug targets. Proteomics technologies are useful for drug discovery. By helping to elucidate the pathomechanism of diseases, proteomics will help the discovery of rational medications that will fit in with the future concept of personalized medicines. A detailed description of various proteomic technologies for drug discovery is given in a special report on proteomics (Jain 2015). A few examples are given here.

Pharmacoproteomics helps to determine the mechanisms of action of bioactive molecules in a systems pharmacology context. In contrast to traditional drug discovery, pharmacoproteomics integrates the mechanism of a drug's action, its side effects including toxicity, and the discovery of new drug targets in a single approach (Hess 2013). This approach facilitates personalized drug discovery.

## *Role of Reverse-Phase Protein Microarray in Drug Discovery*

Reverse-phase protein microarray (RPMA) is a technology platform designed for quantitative, multiplexed analysis of specific phosphorylated, cleaved, or total (phosphorylated and nonphosphorylated) forms of cellular proteins from a limited amount of sample. This class of microarray can be used to interrogate cellular samples, serum or body fluids. RPMA has been applied for translational research and therapeutic drug target discovery (VanMeter et al. 2007). It is particularly suited for oncology. Mapping of protein signaling networks within tumors can identify new targets for therapy and provide a means to stratify patients for individualized therapy. Kinases are important drug targets as such kinase network information could become the basis for development of therapeutic strategies for improving treatment outcome. An urgent clinical goal is to identify functionally important molecular networks associated with subpopulations of patients that may not respond to conventional combination chemotherapy.

## *Dynamic Proteomics for Targeting Disease Pathways*

Dynamic proteomics is the study of dynamics (synthesis, breakdown, transport, storage, etc.) of all proteins in an organism and to translate them into drug discovery, biomarkers and diagnostics. This approach combines the power of liquid chromatography (LC)/mass spectrometry (MS) methods to characterize thousands of proteins in a single sample with kinetic flux analysis for interrogating complex biologic systems to report on those that drive the initiation, progression and reversal of common diseases. Advantages of this approach are:

- Focus on causes rather than symptoms: generating pivotal knowledge for developing blockbuster drugs, by targeting underlying biochemical causes.
- Systems biology approach: insight into intact living systems, rather than simplified models, ensures that drug effects are understood in their intended biological context.
- Reduction of late-stage attrition: early, decision-relevant metrics of drug activity separate winners from losers and reduce losses from later failures.
- Powerful assays of disease state: custom-developed assays to create companion diagnostic tests for personalized medicine.

## *Target Identification and Validation*

The genomics revolution has led to a flood of potential targets but genomic data, by itself, is not be sufficient for validating drug targets. Because most drugs act on proteins, proteins are likely to be more significant therapeutic targets than DNA in

the future. Even the most useful disease biomarkers such as prostate-specific antigen, are proteins. The pathomechanism-based medicine of the future will require input from proteomics for the understanding of how protein pathways link genes to diseases. It is important to understand how the protein function gets deranged in order to design molecules that will correct the aberrant protein.

After a lead molecule is identified, one needs to confirm the efficacy of the drug through the expected mechanism. Proteomics can be used to study the mode of action of drugs by comparing the proteome of the cells in which the drug target has been eliminated by molecular knockout techniques or with small molecule inhibitors believed to act specifically on the same target.

Proteomic techniques enable study of protein expression levels, modifications, location and function in high throughput automated systems. Because proteome analysis can produce comprehensive molecular description of the differences between normal and diseased states, it can be used to compare the effect of candidate drugs on the disease process. Proteomics can be integrated into the drug discovery process along with the genomic and chemical drug discovery. Proteomics may emerge as a powerful approach for directly identifying highly predictive pharmacogenomic biomarkers in blood or other body tissues. Definition and validation of drug targets by proteomics will have the following advantages for drug discovery:

- Fewer dropout compounds in the developmental pipeline
- Rational drug design of compounds with fewer side effects

## Role of Proteomics in Clinical Drug Safety

Clinical chemistry endpoints for routine animal toxicity testing and clinical trial safety monitoring have been used for over 25 years. Drug-induced damage to the liver is the most common type of toxicity that results in a treatment being withdrawn from clinical trials or from further marketing. Similarly, cardiotoxicity is a frequent occurrence in patients undergoing cancer chemotherapy. However, the currently available biomarkers for these common types of drug-induced toxicities have limited sensitivity or predictive value. The proteomic tools available today are enabling us to tap into the wealth of genome sequence information to discover and carefully investigate associations of thousands of proteins with drug-induced toxicities that are now not easily monitored.

### *Toxicoproteomics*

Proteomics can increase the speed and sensitivity of toxicological screening by identifying protein markers of toxicity. Proteomics studies have already provided insights into the mechanisms of action of a wide range of substances, from metals

to peroxisome proliferators. Current limitations involving speed of throughput are being overcome by increasing automation and the development of new techniques. The isotope-coded affinity tag (ICAT) method appears particularly promising.

Toxicoproteomics involves the evaluation of protein expression for the understanding of toxic events. Transcriptional profiling and proteomics are used to compile toxicology predictors. Affinity-based biosensor technology is being investigated to profile lead compound-protein interactions. Immobilized artificial membrane chromatography is being evaluated to predict oral compound absorption. It is expected that these efforts will deliver the tools to annotate screening libraries, hits and leads with quality measures of ADME-tox characteristics. Computational methods will then relate compounds and ADME-tox properties to performance in actual clinical trials. Some examples of application of proteomics in toxicology are given in the following sections.

### Hepatotoxicity

Studies on the rodent liver proteome show that several compounds cause increased proliferation of peroxisomes and liver tumors. Peroxisome proliferators are found to induce protein expression changes as a distinct protein signature. Overdose of acetaminophen causes acute hepatotoxicity in rodents and humans. Experimental evidence suggests that activation of acetaminophen and subsequent formation of protein adducts are involved in hepatotoxicity. Most of the changes caused by acetaminophen occur in a subset of the proteins modified by acetaminophen. Many of the proteins that show changed expression levels are involved in the regulation of mechanisms that are believed to drive acetaminophen-induced hepatotoxicity. Complementary strategies of 2D gel electrophoresis, coupled either with database spot mapping or protein isolation and amino acid sequencing, have successfully identified a subset of proteins from xenobiotic-damaged rodent livers, the expression of which differs from controls.

Lovastatin is a lipid-lowering agent that acts by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key regulatory enzyme in cholesterol biosynthesis. Lovastatin treatment is associated with signs of toxicity as reflected by changes in a heterogeneous set of cellular stress proteins involved in functions such as cytoskeletal structure, calcium homeostasis, protease inhibition, cell signaling or apoptosis. These results present new insights into liver gene network regulations induced by lovastatin and illustrate a yet unexplored application of proteomics to discover new targets by analysis of existing drugs and the pathways that they regulate.

In rat primary hepatocytes exposed to the compounds (acetaminophen, amiodarone, tetracycline and carbon tetrachloride) that are known to induce hepatotoxicity, LDH release and mitochondrial respiration (WST-1 reduction assay) have been used to detect cytotoxicity along with application of proteomic technologies for estimating reliable as well as sensitive biomarkers. Cytotoxicity can be detected

earlier by measuring WST-1 than by measuring LDH release because the reduction of mitochondrial respiration is an expression of earlier toxicity for cellular function, whereas measured increase in LDH release occurs after failure of the cell membrane. Mitochondrial respiration is a useful parameter of cytotoxicity for in vitro hepatotoxicity screening, as cytotoxicity can be detected during an early stage of exposure. In addition to the conventional biomarkers, several protein biomarkers, which relate to oxidative stress and metabolism-regulation, can also be detected. Further comprehensive analysis of defined proteins would be necessary to estimate more sensitive toxicology biomarkers.

**Nephrotoxicity**

An example of dose-related nephrotoxicity is that caused by cyclosporine A which has proven beneficial effects in organ transplantation. Proteomic analysis using 2DE has demonstrated an association between calbinden-D 28 and cyclosporine A-induced nephrotoxicity and is considered to be a marker for this adverse effect. This shows that proteomics can provide essential information in mechanistic toxicology. 2DE and NMR spectrometry was used to study nephrotoxicity in the rat following exposure to puramycin aminonucleoside. Monitoring of proteins in the urine enabled a more detailed understanding of the nature and progression of the proteinuria associated with glomerular nephrotoxicity than was previously possible.

**Neurotoxicity**

Neurotoxicant-induced changes in protein level, function, or regulation could have a detrimental effect on neuronal viability. Direct oxidative or covalent modifications of individual proteins by various chemicals or drugs are likely to lead to disturbance of tertiary structure and a loss of function of neurons. The proteome and the functional determinants of its individual protein components are, therefore, likely targets of neurotoxicant action and resulting characteristic disruptions could be critically involved in corresponding mechanisms of neurotoxicity. A variety of classic proteomic techniques (e.g. LC/tandem mass spectroscopy, 2DG image analysis) as well as more recently developed approaches (e.g. two-hybrid systems, antibody arrays, protein chips, isotope-coded affinity tags, ICAT) are available to determine protein levels, identify components of multiprotein complexes and to detect post-translational changes. Proteomics, therefore, offers a comprehensive overview of cell proteins, and in the case of neurotoxicant exposure, can provide quantitative data regarding changes in corresponding expression levels and/or post-translational modifications that might be associated with neuron injury.

## Applications of Pharmacoproteomics in Personalized Medicine

Examples of clinical applications of proteomic technologies will be given in various chapters dealing with therapeutic areas. Advantages of use of pharmacoproteomics in personalized medicine are:

- Pharmacoproteomics is a more functional representation of patient-to-patient variation than that provided by genotyping.
- Because it includes the effects of post-translational modification, pharmacoproteomics connects the genotype with the phenotype.
- By classifying patients as responders and non-responders, this approach may accelerate personalized drug development.
- Protein biomarkers facilitate integration of diagnostics with therapeutics from development stages to translation into clinical applications.
- Global development of personalized medicine would benefit from pharmacoproteomics technologies and the ways in which they are being applied in Asia-Pacific, particularly India, where there is extensive research investments in "-omics" technologies (Reddy et al. 2011).

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# Chapter 7
# Role of Metabolomics in Personalized Medicine

## Metabolomics and Metabonomics

The human metabolome is best understood by analogy to the human genome, i.e. where the human genome is the set of all genes in a human, the human metabolome is the set of all metabolites in a human. In a systems biology approach, metabolomics provides a functional readout of changes determined by genetic blueprint, regulation, protein abundance and modification, and environmental influence. Metabolomics is the study of the small molecules, or metabolites, contained in a human cell, tissue or organ (including fluids) and involved in primary and intermediary metabolism. By definition, the metabolome should exclude enzymes, genetic material and structural molecules such as glycosaminoglycans, and other polymeric units that are degraded to small molecules but do not otherwise participate in metabolic reactions.

A related term, metabonomics is the use of NMR technology to study metabolomics. According to the Metabolomics Society, "Metabolomics is the study of metabolic changes. It encompasses metabolomics, metabolite target analysis, metabolite profiling, metabolic fingerprinting, metabolic profiling, and metabonomics". Examination of a sample using multiple mass spectrometry-based technologies, nuclear magnetic resonance, integration the data and analysis by proprietary software and algorithms enables faster and more accurate understanding of a disease than previously possible. In spite of the broader scope of metabolomics to include metabonomics, the two terms still continue to be used interchangeably.

The Human Metabolome Database (HMDB) of Canada (http://www.hmdb.ca/) is a freely accessible electronic database containing detailed information about small molecule metabolites found in the human body and contains or links three kinds of data: (1) chemical data; (2) clinical data; and (3) molecular biology/biochemistry data (Wishart et al. 2013). It is meant to be used for applications in metabolomics, clinical chemistry, biomarker discovery and general education. The database contains 41,818 metabolite entries including both water-soluble and lipid soluble metabolites as well as metabolites that would be regarded as either abundant

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or relatively rare. Approximately 5,688 protein sequences are linked to these metabolite entries. Each MetaboCard entry contains >10 data fields with 2/3 of the information being devoted to chemical/clinical data and the other 1/3 devoted to enzymatic or biochemical data. Many data fields are hyperlinked to other databases. The HMDB database supports extensive text, sequence, chemical structure and relational query searches. Four additional databases, DrugBank, T3DB, SMPDB and FooDB are also part of the HMDB suite of databases. DrugBank contains equivalent information on ~1,600 drug and drug metabolites, T3DB contains information on 3,100 common toxins and environmental pollutants, and SMPDB contains pathway diagrams for 440 human metabolic and disease pathways, while FooDB contains equivalent information on ~28,000 food components and food additives.

## Metabolomics Bridges the Gap Between Genotype and Phenotype

In general, phenotype is not necessarily predicted by genotype. The gap between genotype and phenotype is spanned by many biochemical reactions, each with individual dependencies to various influences, including drugs, nutrition and environmental factors. In this chain of biomolecules from the genes to phenotype, metabolites are the quantifiable molecules with the closest link to phenotype. Many phenotypic and genotypic states, such as a toxic response to a drug or disease prevalence are predicted by differences in the concentrations of functionally relevant metabolites within biological fluids and tissues.

A genome-wide association (GWA) study has been carried out with metabolic traits as phenotypic traits (Gieger et al. 2008). Genetically determined variants in metabolic phenotype (metabotype) have been identified by simultaneous measurements of SNPs and serum concentrations of endogenous organic compounds in a human population. Four of these polymorphisms are located in genes. Individuals with polymorphisms in genes coding for well-characterized enzymes of the lipid metabolism have significantly different metabolic capacities with respect to the synthesis of some polyunsaturated fatty acids, the beta-oxidation of short- and medium-chain fatty acids, and the breakdown of triglycerides. Thus, the concept of "genetically determined metabotype" as an intermediate phenotype provides a measurable quantity in the framework of GWA studies with metabolomics and might help to better understand the pathogenesis of common diseases and gene-environment interactions.

Use of this approach to screen previous GWA studies to look for associations between the SNPs of interest and clinical measurements influencing cardiovascular disease, revealed overlap between several SNPs that seem to affect both metabolite biochemistry and clinical outcomes. These metabotypes, in interactions with environmental factors such as nutrition of lifestyle, may influence the susceptibility of an individual for certain phenotypes. For example, there are potential links between

long-chain fatty acid metabolism and attention deficit hyperactivity syndrome. Understanding these connections, in turn, may eventually lead to more targeted nutrition or therapies and more refined disease risk stratification. These could result in a step towards personalized health care and nutrition based on a combination of genotyping and metabolic characterization.

In a multi-"omics" systems biology approach, the metabolome may be the closest biological representation of a clinical trait. Phenomics can be used to fully characterize clinical traits associated with drug therapy, and when combined with metabolomics, common biological pathways can be identified, providing insight into mechanisms of efficacy and safety (Monte et al. 2014). This approach has the potential to eliminate drug therapy that will either be ineffective or unsafe in specific subsets of patients.

## Metabolomics, Biomarkers and Personalized Medicine

Metabolomics has used to identify biomarkers for disease as well as to identify off-target side effects in marketed drugs and new chemical entities in development. Compared to ~19,000 genes and ~1 million proteins, there are only 2,500 metabolites (small molecules). Their limited number enables an easier, more quantitative method of analysis. Examination of a sample using multiple mass spectrometry (MS)-based technologies, integration the data and analysis by proprietary software and algorithms enables faster and more accurate understanding of a disease than previously possible. Plasma samples obtained from patients can be analyzed for signatures of neurodegenerative disorders by measuring the spectrum of biochemical changes and mapping these changes to metabolic pathways. This technology can be applied to discover biomarkers for diabetic nephropathy in type 1 diabetes. Metabolomic profiling should be included in personalized medicine.

Within the last few years, metabolomics has developed into a technology that complements proteomics and transcriptomics. In combination with techniques for functional analysis of genes, it is hoped that a holistic picture of metabolism can be formed. In addition to the genome analysis and proteome analyses, the exhaustive analysis of metabolites is important for a comprehensive understanding of cellular functions because the dynamic behavior of metabolites cannot be predicted without information regarding metabolome.

In view of the chemical and physical diversity of small biological molecules, the challenge remains of developing protocols to gather the whole 'metabolome'. No single technique is suitable for the analysis of different types of molecules, which is why a mixture of techniques has to be used. In the field of metabolomics, the general estimations of the size and the dynamic range of a species-specific metabolome are at a preliminary stage. Metabolic fingerprinting and metabonomics with high sample throughput but decreased dynamic range and the deconvolution of individual components achieve a global view of the in vivo dynamics of metabolic

networks. The technologies used include NMR, direct infusion mass spectrometry, and/or infrared spectroscopy. Gas chromatography (GC)-MS and LC-MS technology achieve a lower sample throughput but provide unassailable identification and quantitation of individual compounds in complex samples.

Major steps forward in these technologies have made it possible to match specific demands with specific instruments and novel developments in the performance of mass analyzers or mass spectrometers (MS). Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR) is a type of MS for determining the mass-to-charge ratio of ions based on the cyclotron frequency of the ions in a fixed magnetic field and rapidly measures many metabolites in a single experiment.

However, it is important to note that each type of technology exhibits a bias towards certain compound classes, mostly due to ionization techniques, chromatography and detector capabilities. GC-MS has evolved as an imperative technology for metabolomics due to its comprehensiveness and sensitivity. The coupling of GC to time-of-flight (TOF) mass analyzers is an emerging technology. High scan rates provide accurate peak deconvolution of complex samples. GC-TOF-MS capabilities provide an improvement over conventional GC-MS analysis of ultracomplex samples, which is particularly important for the metabolomics approach. Ultracomplex samples contain hundreds of co-eluting compounds that vary in abundance by several orders of magnitude. Thus, accurate MS deconvolution and a broad linear dynamic range represent indispensable prerequisites for high quality spectra and peak shapes. Modern GC-TOF-MS applications and incorporated MS deconvolution algorithms fulfill these requirements. The advantages of metabolomics technologies are:

- Ability to analyze all bodily fluids such as blood, CSF and urine as well as cultured or isolated cells and biopsy material.
- High throughput capability enabling simultaneous monitoring of biological samples
- Analysis of multiple pathways and arrays of metabolites simultaneously from microliter sample quantities.

## *Urinary Profiling by Capillary Electrophoresis*

Metabolomic approaches have become particularly important for discovery of biomarkers in urine. The analytical technology for urinary profiling must be efficient, sensitive and offer high resolution. Until recently these demands were commonly met by HPLC-MS, GC-MS and NMR. The analytical armory for urinary profiling has now been extended to include cyclodextrin-modified micellar electrokinetic capillary chromatography (CD-MECC), which enables highly cost-effective, rapid and efficient profiling with minimal sample volume and preparation requirements. The CD-MECC profiles typically show separation for >80 urinary metabolites. These profiles have been visualized using novel advanced pattern recognition tools. Visualization of pattern changes has been achieved through development of the

novel ACE (Automated Comparison of Electropherograms) software which not only removes errors due to baseline shifts but also allows for rapid reporting of semiquantitative profile differences. The method has been applied in investigation of biomarkers characteristic of alcoholics or Down's syndrome persons.

## *Lipid Profiling*

Modern medicine has come to rely on a small suite of single biomarkers, such as plasma cholesterol or triglycerides, to assess the risk of certain diseases. However, such single-biomarker assessments overlook the inherent complexity of metabolic disorders involving hundreds of biochemical processes. Assessing the full breadth of lipid metabolism is what drives the field of lipomic profiling. However, unlike the other "-omics" technologies, in which only a small portion of the genes or proteins is known, lipid metabolic pathways are well characterized. Another limitation of "-omics" technologies is that they produce so many false positive results that it is difficult to be sure that findings are valid. Metabolomics is not immune to this problem but, when practiced effectively, the technology can reliably produce knowledge to aid in decision making. Focused metabolomics platforms, which restrict their target analytes to those measured well by the technology, can produce data with properties that maximize sensitivity and minimize the false discovery problem. The most developed focused metabolomics area is lipid profiling. TrueMass® (Lipomic Technologies) analysis produces lipomic profiles – comprehensive and quantitative lipid metabolite profiles of biological samples. With TrueMass, Lipomics measures hundreds of lipid metabolites from each small quantity of tissue, plasma or serum sample. Because the resulting data are quantitative, TrueMass data can be seamlessly integrated with pre-existing or future databases.

Data-dependent acquisition of MS/MS spectra from lipid precursors enables emulation of the simultaneous acquisition of an unlimited number of precursors and neutral loss scans in a single analysis. This approach takes full advantage of rich fragment patterns in tandem MS of lipids and enables their profiling by complex scans, in which masses of several fragment ions are considered within a single logical framework. No separation of lipids is required, and the accuracy of identification and quantification is not compromised, compared to conventional precursor and neutral loss scanning.

## *Role of Metabolomics in Biomarker Identification and Pattern Recognition*

Metabolomics research has increased significantly over recent years due to advances in analytical measurement technology and the advances in pattern recognition software enabling one to visualize changes in levels of hundreds or even thousands of

chemicals simultaneously. Multivariate metabolomic and proteomic data and time-series measurements can be combined to reveal protein-metabolite correlations. Different methods of multivariate statistical analysis can be explored for the interpretation of these data. The discrimination of the samples enables the identification of novel components. These components are interpretable as inherent biological characteristics.

Biomarkers that are responsible for these different biological characteristics can easily be classified because of the optimized separation using independent components analysis and an integrated metabolite-protein dataset. Evidently, this kind of analysis depends strongly on the comprehensiveness and accuracy of the profiling method, in this case metabolite and protein detection. Assuming that the techniques will improve, more proteins and metabolites can be identified and accurately quantified, the integrated analysis will have great promise.

### *Validation of Biomarkers in Large-Scale Human Metabolomics Studies*

A strategy for data processing and biomarker validation has been described in a large metabolomics study that was performed on 600 plasma samples taken at four time points before and after a single intake of a high fat test meal by obese and lean subjects (Bijlsma et al. 2006). All samples were analyzed by a LC-MS lipidomic method for metabolic profiling. Such metabolomics studies require a careful analytical and statistical protocol. A method combining several well-established statistical methods was developed for processing this large data set in order to detect small differences in metabolic profiles in combination with a large biological variation. The strategy included data preprocessing, data analysis, and validation of statistical models. After several data preprocessing steps, partial least-squares discriminate analysis (PLS-DA) was used for finding biomarkers. To validate the found biomarkers statistically, the PLS-DA models were validated by means of a permutation test, biomarker models, and noninformative models. Univariate plots of potential biomarkers were used to obtain insight in up- or down-regulation.

## Pharmacometabonomics

A major factor underlying inter-individual variation in drug effects is variation in metabolic phenotype, which is influenced not only by genotype but also by environmental factors such as nutritional status, the gut microbiota, age, disease and the co- or pre-administration of other drugs. Thus, although genetic variation is clearly important, it seems unlikely that personalized drug therapy will be enabled for a wide range of major diseases using genomic knowledge alone. Metabolite patterns

that are characteristic of the individual can be used to diagnose diseases, predict an individual's future illnesses, and their responses to treatments.

The principle of pharmacometabonomics has been demonstrated in humans by showing a clear connection between an individual's metabolic phenotype, in the form of a predose urinary metabolite profile, and the metabolic fate of a standard dose of the widely used analgesic acetaminophen (Clayton et al. 2009). Predose and postdose urinary metabolite profiles were determined by $^{1}$H NMR spectroscopy. The predose spectra were statistically analyzed in relation to drug metabolite excretion to detect predose biomarkers of drug fate and a human-gut microbiome cometabolite predictor was identified. Thus, the investigators found that individuals having high predose urinary levels of p-cresol sulfate had low postdose urinary ratios of acetaminophen sulfate to acetaminophen glucuronide. They conclude that, in individuals with high bacterially mediated p-cresol generation, competitive O-sulfonation of p-cresol reduces the effective systemic capacity to sulfonate acetaminophen. Given that acetaminophen is such a widely used and seemingly well-understood drug, this finding provides a clear demonstration of the immense potential and power of the pharmacometabonomic approach. However, many other sulfonation reactions are expected to be similarly affected by competition with p-cresol and these finding also has important implications for certain diseases as well as for the variable responses induced by many different drugs and xenobiotics. It is proposed that assessing the effects of microbiome activity should be an integral part of pharmaceutical development and of personalized health care. Furthermore, gut bacterial populations might be deliberately manipulated to improve drug efficacy and to reduce adverse drug reactions. Pharmacometabonomics could be used to preselect volunteers at key stages of the clinical trials. This would enable stratification of subjects into cohorts, which could minimize the risk of adverse events, or focus on those individuals with a characteristic disease phenotype for assessment of efficacy.

## Metabonomic Technologies for Toxicology Studies

Metabonomics studies demonstrate its potential impact in the drug discovery process by enabling the incorporation of safety endpoints much earlier in the drug discovery process, reducing the likelihood (and cost) of later stage attrition.

Global metabolic profiling (metabonomics/metabolomics) has shown particular promise in the area of toxicology and drug development. A metabolic profile need not be a comprehensive survey of composition, nor need it be completely resolved and assigned, although these are all desirable attributes. For the profile to be useful across a range of problems, however, it must be amenable to quantitative interpretation and it should be relatively unbiased in its scope. In addition to explicit quantification of individual metabolites, analytical profiles such as NMR spectra are effectively functions of the concentrations of the constituents of the sample and hence can be handled directly as metabolic profiles. A further requirement for the

platform used to generate profiles is that the analytical variation introduced postcollection be less than the typical variation in the normal population of interest, so as not to reduce significantly the opportunity to detect treatment/group-related differences. Fulfilling this condition is very dependent on the actual system and question in hand and is probably best tested in each new application.

In both preclinical screening and mechanistic exploration, metabolic profiling can offer rapid, noninvasive toxicological information that is robust and reproducible, with little or no added technical resources to existing studies in drug metabolism and toxicity. Extended into the assessment of efficacy and toxicity in the clinic, metabonomics may prove crucial in making personalized therapy and pharmacogenomics a reality.

## Metabonomics/Metabolomics and Personalized Nutrition

Metabometrix (London, UK) specializes in metabonomics. The company believes that it is possible to profile metabolic diseases before symptoms appear. Metabonomic testing is important in obesity/metabolic syndromes, in which several metabolic pathways interact to produce symptoms and could be an important guide to select diets and exercise programs tailored to metabolic states.

It is considered desirable to establish a human "metabonome" parallel to human genome and proteome but it will be a formidable undertaking requiring analysis of at least half a million people. Some projects are examining metabonomic patterns in series of patients with metabolic syndromes and comparing them with normal people. Other studies are examining how a person's unique metabonomic profile can be used as a guide to personalize diet and exercise regimens for obesity.

It is now possible to measure hundreds or thousands of metabolites in small samples of biological fluids or tissues. This enables assessment of the metabolic component of nutritional phenotypes and will enable individualized dietary recommendations. The relation between diet and metabolomic profiles as well as between those profiles and health and disease needs to be established. Appropriate technologies should be developed and that metabolic databases are constructed with the right inputs and organization. Moreover, social implications of these advances and plan for their appropriate utilization should be considered.

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# Chapter 8
# Non-genomic Factors in the Development of Personalized Medicine

## Introduction

Besides genomics other omics, epigenomic and non-genomic factors and biotechnologies have contributed to the development of personalized medicine. Although personalized medicine is considered to be mostly based on pharmacogenomics, a number of other factors that vary among individuals and should be considered are:

- Identification of subpopulation of patients best suited for an existing drug
- New drug design for a specific sub-population of patients
- Use of an individual patient's cells or tissues for biological therapies
- Cytomics: analysis of disease at single cell level.
- Pharmacoepigenomics
- In case of cancer, sub-classification of type of cancer best suited for a drug
- Drug dose adjusted to metabolic status of an individual
- Drug administration at the right stage in the course of the disease for optimal benefit.
- Drug administration at the right time of the day taking into consideration the relation of the disease manifestations according to diurnal rhythms, e.g. blood pressure values in hypertensive patients fluctuate according to time of the day.
- Follow-up of therapy with a biomarker or diagnostic assays

Some of the factors have been discussed in other chapters. Other factors are discussed briefly in this section. Proteomics and metabolomics are topics of separate chapters. Some of the other non-genomic factors will be considered in this chapter. Among biotechnologies, nanobiotechnology has made important contributions to the development of personalized medicine.

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## Circadian Rhythms and Personalized Medicine

Various physiological and metabolic processes in the human body including sleep-wake cycles, metabolism, heart rate, blood pressure, body temperature, renal activity, and endocrine secretions fluctuate in a daily manner. They are attributed to circadian rhythms, which are endogenous self-sustained oscillations with a period of ~24 h. Circadian rhythms are coordinated by a biological clock situated in the suprachiasmatic nuclei (SCN) of the hypothalamus. These rhythms persist under constant environmental conditions, demonstrating their endogenous nature. Some rhythms can be altered by disease. Several clock genes and clock-controlled transcription factors regulate, at least in part, gene expression in central and/or peripheral clocks. However, virtually all of our 35 trillion body cells also possess their own clocks, which are indistinguishable from those operative in SCN neurons (Bollinger and Schibler 2014).

The rhythms of disease and pharmacology can be taken into account to modulate treatment over the 24-h period, i.e. chronotherapy. The term "chronopharmacology" is applied to variations in the effect of drugs according to the time of their administration during the day. "Chronopharmacokinetics" is defined as the predictable changes observed in the plasma levels of drugs and in the parameters used to characterize the pharmacokinetics of a drug. Half-life of a drug can vary as a function of the hour of administration.

The efficacy and/or toxicity of drugs depend on an individual's body time (BT). Drug administration at the appropriate BT can improve the outcome of pharmacotherapy by maximizing potency and minimizing the toxicity of the drug, whereas drug administration at an inappropriate BT can induce severe side effects. Information obtained by detection of individual BT via a single-time-point assay can be exploited to maximize potency and minimize toxicity during drug administration and thus will enable highly optimized medication. Genome-wide gene expression analyses using high-density DNA microarrays have identified clock-controlled genes. BT based on expression profiles of time-indicating genes reflects the endogenous state of the circadian clock. In clinical situations, methods for BT detection should be applicable for populations with heterogeneous genetic backgrounds.

A molecular timetable is consists of >100 time-indicating genes, whose gene expression levels can represent internal BT. A study in mice has shown that 43 % of genes follow a daily schedule in at least 1 of the 12 organs profiled, and that 56 of the 100 best-selling drugs in the US target products of genes whose expression cycles oscillate according to circadian rhythms in clinically relevant organs (Zhang et al. 2014). Using samples taken every 2 h, the researchers probed mRNA using microarrays and quantified expression of ~20,000 protein-coding genes. They also used RNA-sequencing on organs sampled every 6 h, which enabled them to profile the cycling of non-coding RNA. Most of these genes were previously recognized clock genes that are responsible for the keeping the body's internal daily rhythm. There also seemed to be gene-expression "rush hours," just before dawn and dusk.

The body needs a completely different set of genetic programs to perform activities than it does for sleep and restoration.

The knowledge of expression of gene relevant to circadian rhythms might enable identification of drugs whose efficacy and side effects are most likely to be affected by time of administration. The antihypertensive drug Diovan and Ritalin used for attention deficit hyperactivity disorder have half-lives of <6 h, which means that if they are administered at the wrong time, they might break down before having a chance to fully engage with their targets. The timing of drug administration could also explain why some persons in clinical trials seem to respond to a medication while others do not. It should be noted that most studies on mice are performed during the day, when the animals should be asleep. Humans most often take drugs in the daytime, during their wake cycle, which could explain why drug effects can fail to translate from mice to humans.

Already there are several examples of how chronotherapy has improved management of several diseases. A clinical trial of low-dose glucocorticoid chronotherapy added to disease-modifying treatment produces rapid and relevant improvements in signs and symptoms of rheumatoid arthritis with better results than conventional glucocorticoid administration (Buttgereit et al. 2013). An oral prednisone tablet was designed to release the drug at 2 AM, the time of best efficacy. This formulation can be ingested before going to sleep, so that the patient's sleep does not have to be interrupted. Model-based personalized circadian drug delivery aims at jointly improving tolerability and efficacy of anticancer drugs based on the circadian timing system of individual patients, using dedicated circadian biomarker and drug delivery technologies (Ortiz-Tudela et al. 2013). In a comparison of overall survival of male patients with colorectal cancer there was a clear advantage of applying a chronomodulated regimen of a combination of 5-fluorouracil, leucovorin and oxaliplatin over a conventional infusion of these drugs. However, no statistically significant overall survival difference between these treatments was observed for the time-adapted treatment of female patients. More clinical studies are required to assess the virtues of chronotherapeutic regimens in fighting cancer and other diseases.

## Environmental Factors in Disease

Environmental factors can precipitate a disease in an individual genetically predisposed to it. Most differences in responses to drugs in human are multifactorial, caused by genetic plus environmental factors and this is an argument for the broader approach of personalized medicine rather than the limited approach of pharmacogenetics or pharmacogenomics. Some adverse drug reactions are caused by interaction of the drugs with environmental toxins, infectious organisms or dietary constituents. Therefore, prescription of drugs based genotype tests to individuals considered safe to receive the drugs, may not completely eliminate the possibility of such a reaction. A patient matched to a drug on the basis of a genotyping test may

not necessarily respond to it. Although there is considerable improvement in safety and efficacy of a limited number of drugs available now in combination with diagnostics, investigation of environmental factors must continue to identify other factors, which will vary from one patient to another and would still come under the scope of personalized medicine.

A Committee on Environmental Exposure Technology Development of the NIH has identified a "toolbox" of methods such as biosensors and toxicogenomics for measuring external (environmental) and internal (biologic) exposure to assess human behaviors that influence the likelihood of exposure to environmental agents at a personal level (Weis et al. 2005). The aim is to understand complex human diseases using an integrated approach to exposure assessment to define particular exposure-disease relationships and the interaction of genetic and environmental factors in disease occurrence. Improved methods for exposure assessment will result in better means of monitoring and personalized intervention and prevention programs.

Most of the focus of research on causes of human diseases has been on genome-wide association studies (GWAS). In 2014, NIH is funding research at Center for Biomedical Informatics of Harvard Medical School for examining data from environment-wide association studies (EWAS) and GWAS to find out if and how environmental and genetic factors act together to increase disease risk. Bioinformatics methods will be applied to epidemiological data to identify interacting environmental exposures and genetic variants in cardiovascular risk traits, such as high blood pressure, and then to design an epidemiological study to test the clinical utility of using these tools to predict risk for coronary heart disease.

## *Human Intestinal Microflora*

The human intestinal microflora is composed of $10^{13}$ to $10^{14}$ microorganisms whose collective genome (microbiome) contains at least 100 times as many genes as the human genome. A study has analyzed approximately 78 million base pairs of unique DNA sequence and 2062 PCR-amplified 16S ribosomal DNA sequences obtained from the fecal DNAs of two healthy adults, one male and one female, who had not received any antibiotic in the past (Gill et al. 2006). Using metabolic function analyses of identified genes, the human genome was compared with the average content of previously sequenced microbial genomes. The gut microbiome has significantly enriched metabolism of glycans, amino acids, and xenobiotics; methanogenesis; and 2-methyl-d-erythritol 4-phosphate pathway-mediated biosynthesis of vitamins and isoprenoids. This study concludes that humans are super organisms whose metabolism represents an amalgamation of microbial and human attributes. Without understanding the interactions between human and microbial genomes, it is impossible to obtain a complete picture of human biology. The next frontier in the field of genetic research called metagenomics. This has implications for clinical diagnosis and treatment of many human diseases. With the knowledge gained in this area, one

can use biomarkers to identify the bacterial population of the individual. Physicians can then manipulate the population of bacteria to consistent with optimal health of an individual. Such an analysis would also identify bacteria that are resistant to certain antibiotics, and enable selection of the appropriate antibiotic for a patient. In the future, healthy individuals could undergo a metagenomic analysis of their gut to determine their immune status and susceptibility to certain diseases. Such an analysis may enable assessment of the effects of age, diet and diseases such as inflammatory bowel disease, cancer and obesity on the microbial flora of the distal gut in persons living in different environments with different dietary habits.

### *Metabolic Interactions of the Host and the Intestinal Microflora*

The mammalian gut microbes interact extensively with the host through metabolic exchange and co-metabolism of substrates. They influence both the biochemistry and immune system of the host. Their interactions with the host are poorly understood, but might be implicated in the etiology of many human diseases. The gut microflora may have effects that cannot be predicted from the patient's genome alone. Currently, when developing a new drug little factors such as the microflora are not taken into consideration but this may need to change. Many species produce compounds that switch on detoxification enzymes in the liver and certain microbial metabolites are necessary players in human metabolic pathways. Because the gut microbes influence the disposition, fate and toxicity of drugs in the host, an appropriate consideration of individual human gut microbial activities will be a necessary part of future personalized health-care paradigms. Several companies including Pfizer and Bristol-Myers-Squibb are developing metabonomic technology that identifies metabolomic patterns that predict both a drug's toxicity and the biochemical pathway involved. Such data need to be integrated statistically with information from other "omics" such as proteomics and transcriptomics for a complete picture of the drug action.

The influence of gut microbiota on the toxicity and metabolism of hydrazine has been investigated in germ-free and 'conventional' Sprague Dawley rats using 1H NMR based metabonomic analysis of urine and plasma (Swann et al. 2009). Toxicity was more severe in germ-free rats compared with conventional rats for equivalent exposures indicating that bacterial presence altered the nature or extent of response to hydrazine and that the toxic response can vary markedly in the absence of a functional microbiome.

## Epigenomics and Personalized Medicine

Epigenomics is the study of epigenetic modifications of the genetic material of a cell – the epigenome (Russell 2010). The epigenome consists of chemical compounds that modify, or mark, the genome in a way that tells it what to do, where to

do it and when to do it. The marks, which are not part of the DNA itself, can be passed on from cell to cell as cells divide, and from one generation to the next. Lifestyle and environmental factors can expose a person to chemical tags that change the epigenome. The most characterized epigenetic modifications are DNA methylation and histone modification.

As part of its Roadmap for Medical Research, the NIH plans to develop a map of the epigenomic marks that occur on the human genome. The progress can be followed on the web site: http://commonfund.nih.gov/epigenomics. In addition to genomics, knowledge of epigenomics is essential for understanding the pathogenesis of several diseases, particularly cancer, where a combination of alterations in the genome as well as the epigenome promote the malignant transformation. The combination of mutations, structural variations and epigenetic alterations differs between each tumor, making individual diagnosis and treatment strategies necessary for a personalized approach to management (Schweiger et al. 2013).

### *Genetics vs. Epigenetics*

The sequence of the four nucleotides of the genetic code is compared to an indelible ink that, with rare exceptions, is faithfully transcribed from cell to cell and from generation to generation. The epigenetic code lies on top of this and is represented by methyl groups added to the DNA base cytosine, as well as covalent changes in histone proteins around which the DNA is coiled. This epigenetic information is more like a code written in pencil in the margins around the DNA (Gosden and Feinberg 2007). Regulation of gene expression by genetics involves a change in the DNA sequence, whereas epigenetic regulation involves alteration in chromatin structure and methylation of the promoter region. DNA methylation represents an epigenetic means of inheritance without associated DNA sequence alterations. The role of epigenetics in the etiology of human disease is increasingly recognized with the most obvious evidence found for genes subject to genomic imprinting.

## Cytomics as a Basis for Personalized Medicine

Cytomics is the structural and functional information is obtained by molecular cell phenotype analysis of tissues, organs and organisms at the single cell level by image or flow cytometry in combination with bioinformatic knowledge extraction concerning nuclei acids, proteins and metabolites (cellular genomics, proteomics and metabolomics) as well as cell function parameters like intracellular pH, transmembrane potentials or ion gradients. In addition, differential molecular cell phenotypes between diseased and healthy cells provide molecular data patterns for (i) predictive medicine by cytomics or for (ii) drug discovery purposes using reverse engineering of the data patterns by biomedical cell systems biology. Molecular pathways can be

explored in this way including the detection of suitable target molecules, without detailed a priori knowledge of specific disease mechanisms. This is useful during the analysis of complex diseases such as infections, allergies, rheumatoid diseases, diabetes or malignancies. The top-down approach reaching from single cell heterogeneity in cell systems and tissues down to the molecular level seems suitable for a human cytomics project to systematically explore the molecular biocomplexity of human organisms. The analysis of already existing data from scientific studies or routine diagnostic procedures will be of immediate value in clinical medicine, for example as personalized therapy by cytomics (Valet 2005).

## Contributions of Nanobiotechnology to Personalized Medicine

Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e., at the level of atoms, molecules, and supramolecular structures. It is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured in nanometers (a nanometer is one billionth of a meter i.e., $10^{-9}$ m). Nanobiotechnology is the application of nanotechnology in life sciences and is the subject of a special report (Jain 2015). Applications in medicine are described in a book on nanomedicine (Jain 2012). Applications in personalized management of cancer are described in Chap. 10 and those in cardiovascular disorders in Chap. 14.

### *Role of Nanobiotechnology in Molecular Diagnostics*

Application of nanobiotechnology in molecular diagnostics is called nanodiagnostics and it will improve the sensitivity and extend the present limits of molecular diagnostics (Jain 2005, 2007). Because DNA, RNA, protein and their functional subcellular scaffolds and compartments, are in the nanometer scale, the potential of single molecule analysis approach would not be fully realized without the help of nanobiotechnology. Advances in nanotechnology are providing nanofabricated devices that are small, sensitive and inexpensive enough to facilitate direct observation, manipulation and analysis of single biological molecule from single cell. This opens new opportunities and provides powerful tools in the fields such as genomics, proteomics, molecular diagnostics and high throughput screening.

Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. It seems quite likely that there will be numerous applications of inorganic nanostructures in biology and medicine as markers. Given the inherent nanoscale of receptors, pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive and more

flexible when certain nanoscale particles are put to work as tags or labels. Nanoparticles are the most versatile material for developing diagnostics. Nanotechnology has potential advantages in applications in point-of-care (POC) diagnosis: on patient's bedside, self-diagnostics for use in the home, integration of diagnostics with therapeutics and for the development of personalized medicines.

Nanomaterials can be assembled into massively parallel arrays at much higher densities than is achievable with current sensor array platforms and in a format compatible with current microfluidic systems. Currently, quantum dot technology is the most widely employed nanotechnology for diagnostic developments. Among the recently emerging technologies, cantilevers are the most promising.

### Cantilevers for Personalized Medical Diagnostics

An innovative method for the rapid and sensitive detection of disease- and treatment-relevant genes is based on cantilevers. This method detects active genes directly by measuring their transcripts (mRNA), which represent the intermediate step and link to protein synthesis. Short complementary nucleic acid segments (sensors) are attached to silicon cantilevers which are 450 nm thick and therefore react with extraordinary sensitivity. Binding of targeted gene transcripts to their matching counterparts on cantilevers results in mechanical bending that can be optically measured. Differential gene expression of the gene 1-8U, a potential biomarker for cancer progression or viral infections, can be observed in a complex background. The measurements provide results within minutes at the picomolar level without target amplification, and are sensitive to base mismatches. An array of different gene transcripts can even be measured in parallel by aligning appropriately coated cantilevers alongside each other like the teeth of a comb. This method complements current molecular diagnostic techniques such as the gene chip and real-time PCR. It could be used as a real-time sensor for continuously monitoring various clinical parameters or for detecting rapidly replicating pathogens that require prompt diagnosis. These findings qualify the technology as a rapid method to validate biomarkers that reveal disease risk, disease progression or therapy response. This technology complements and extends current DNA and protein microarray methods, because nanomechanical detection requires no labels, optical excitation, or external probes and is rapid, highly specific, sensitive, and portable. This will have applications in genomic analysis, proteomics and molecular diagnostics. Cantilever arrays have potential as a tool to evaluate treatment response efficacy for personalized medical diagnostics.

### Nanobiotechnology for Therapeutics Design and Monitoring

Current therapeutic design involves combinatorial chemistry and system biology-based molecular synthesis and bulk pharmacological assays. Therapeutics delivery is usually non-specific to disease targets and requires excessive dosage. Efficient therapeutic discovery and delivery would require molecular level understanding of

the therapeutics-effectors (e.g. channels and receptors) interactions and their cell and tissue responses. A multidimensional nanobiotechnology-based approach to personalized medicine starts with scanning probe techniques, especially atomic force microscopy (AFM) to identify potential targets for drug discovery (Lal and Arnsdorf 2010). AFM can be integrated with nanocarriers and implantable vehicles for controlled delivery. Characterization of nanocarrier-based drug delivery can enable high efficiency of in vivo or topical administration of a small dosage of therapeutics. High-throughput parallel nanosensors, comprising integrated cantilevered microarrays, total internal reflection fluorescence (TIRF) microscopy, microfluidics and nanoelectronics, can be used for rapid diagnosis of diseases, detection of biomarkers as well as for therapeutics design. Therapeutic efficacy can be assessed by monitoring biomechanics. These will be important contributions to personalized medicine.

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# Chapter 9
# Personalized Biological Therapies

## Introduction

Historically blood transfusion and organ transplantation were the first personalized therapies as they were matched to the individuals. Some cell therapies that use patient's own cells are considered to be personalized medicines particularly vaccines prepared from the individual patient's tumor cells. More recently recombinant human proteins might provide individualization of therapy. The number of biotechnology-based therapeutics introduced in medical practice is increasing along with their use in a personalized manner (Jain 2012).

## Recombinant Human Proteins

There are a large number of therapeutic proteins approved for clinical use and many more are undergoing preclinical studies and clinical trials in humans. Most of them are human or 'humanized' recombinant molecules. Virtually all therapeutic proteins elicit some level of antibody response, which can lead to potentially serious side effects in some cases. Therefore, immunogenicity of therapeutic proteins is a concern for clinicians, manufacturers and regulatory agencies. In order to assess immunogenicity of these molecules, appropriate detection, quantitation and characterization of antibody responses are necessary. Immune response to therapeutic proteins in conventional animal models has not been, except in rare cases, predictive of the response in humans. In recent years there has been a considerable progress in development of computational methods for prediction of epitopes in protein molecules that have the potential to induce an immune response in a recipient. Such tools are already being applied in the development of therapeutic proteins. It is expected that computer driven prediction followed by in vitro and/or in vivo testing of any potentially immunogenic epitopes will help in avoiding, or at least minimizing, immune responses to therapeutic proteins. It is possible to develop

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recombinant proteins in combination with diagnostic tests to limit their use to patients where they are least likely to induce immune reactions.

Another approach to protein therapy is in vivo production of proteins by genetically engineered cells where the delivery of proteins can be matched to the needs of the patient and in vivo production and controlled delivery might reduce adverse effects.

## Therapeutic Monoclonal Antibodies

Compared with small-molecule drugs, antibodies are very specific and are less likely to cause toxicity based on factors other than the mechanism of action. Orally available small molecules have many targets but they may also hepatotoxic and are involved in drug-drug interactions. They may interfere with cytochrome P-450. From the point of view of a clean safety profile, antibodies are extremely attractive. They can be designed to be very specific with high affinity for the target.

Antibodies have for many decades been viewed as ideal molecules for cancer therapy. Genetic engineering of antibodies to produce chimeric or humanized monoclonal antibodies (hMAbs) has greatly advanced their utility in molecular targeting therapies. These will be described in more detail in the section on personalized cancer therapy in Chap. 8. Many molecular biological and immunological studies have revealed the targeting properties of the host immune system and the biological mechanisms of cancer cells for a more specific anticancer effect. Many clinical trials of MAbs as a single agent, or in combination protocol with current standard chemotherapy or immunoconjugates have shown promise in the treatment of specific diseases. Furthermore, novel MAb designs and improved understanding of the mode of action of current MAbs lend great hope to the future of this therapeutic approach. The accumulating results from many basic, clinical and translational studies may lead to more individualized therapeutic strategies using these agents directed at specific genetic and immunologic targets.

## Cell Therapy

Cell therapy is the prevention or treatment of human disease by the administration of cells that have been selected, multiplied and pharmacologically treated or altered outside the body (ex vivo). The aim of cell therapy is to replace, repair or enhance the function of damaged tissues or organs. The cells used can originate from the patient or from a donor or from another species. Other sources include cell lines and cells from patients' tumors to make cancer vaccines. Cells can be encapsulated in selectively permeable membranes that block entry of immune mediators but allow outward diffusion of active molecules produced by the cells. Genetic engineering of

cells is part of ex vivo gene therapy. The cells may be introduced by various routes into the body and selectively implanted at the site of action. Cell therapy is described in detail in a special report (Jain 2015a).

## *Autologous Tissue and Cell Transplants*

The term transplantation, used mostly for organ transplants in the past, is now also used for cells transplanted from one individual to another. Cells can be used to restore lost functions of organ, i.e. organ repair instead of organ replacement. Problems associated with transplantation include organ rejection requiring immunosuppressive therapy. Problems of rejection of grafted cells can be solved by using the patient's own cells (autologous) and encapsulating cells from other sources.

## *Stem Cells*

Stem cells are cells in the embryo and the adult human body that retain the capability of making a range of other cell types. In the embryo, these cells are the starting point for the development of the complete human being. In the adult, stem cells are one of the resources for repair and renewal of cells/tissues and may be used for personalized therapy. Embryonic stem cells (ESCs) are continuously growing cell lines of embryonic origin derived from the pluripotent cells of the inner cell mass or epiblast of the mammalian embryo. They may give rise to any cell type but not to an independent organism. Adult stem cells of the individual patient are more suitable for personalized therapy. Availability of technologies to derive induced pluripotent stem cells (iPSCs) from adult somatic cells will enhance the potential of personalized cell-based therapy.

### Induced Pluripotential Stem Cells for Personalized Cell Therapy

Induced pluripotential stem cell (iPSC) technology has raised hopes of treating various human diseases that were previously considered untreatable and enabled personalized medicine without ethical issues and immunological rejection that may occur with human ESC (hESC) transplantation. iPSCs enable disease modeling that mimics human pathological processes rather than tests using conventional animal models and cell lines. It is possible to routinely generate iPSC from patient-specific cell sources, such as skin fibroblast, hair follicle cells, and blood samples. iPSCs resemble hESCs for their ability to regenerate tissue and even a full organism. iPSC provide a better choice for cell-based therapy. Tissue memory containing iPSC from mature leukocytes may be beneficial for curing cancer and infectious diseases (Kim 2014).

**Role of Stem Cells Derived from Unfertilized Embryos**

Using unfertilized human oocytes as a source for stem cell is less controversial than using fertilized embryos; it avoids the ethical concerns surrounding human ESC research. Without the contribution from a sperm, the oocyte has a unique advantage of homozygosity, which renders its derivatives less immunogenic and provides a broader match with different MHC phenotypes. In addition, stem cells derived from unfertilized oocytes could also be selected for homozygosity of a drug response gene, a disease gene, or a cancer gene from a female carrier and, therefore, could provide a model and business rationale for drug testing and drug discovery. For example, a collection of stem cells homozygous for different drug metabolizing gene variants could be used to prescreen a drug for its prospective toxicity and efficacy in the population. A cancer progression model can be established by differentiating stem cells homozygous for a cancer gene to the cancer tissue types, leading to the identification of biomarkers of cancer progression and drugs for cancer prevention. Furthermore, these homozygous stem cells could be used in facilitating linkage studies and in verifying the function of a SNP.

## *Cloning and Personalized Cell Therapy*

Cloning is the procedure used to create a cell or organism that is genetically identical to an existing cell or organism. The underlying biological mechanism of cloning is the reprogramming of the nuclei of specialized adult cells to become the nuclei of new embryonic cells. Cloning cells in the laboratory is a routine procedure used to produce life-saving therapeutic proteins such as human insulin for the treatment of diabetes. Potential further applications of cloning can improve treatments for illnesses stroke, Parkinson's disease and heart disease. Human therapeutic cloning provides a potentially limitless source of cells for cell therapy and tissue engineering. Cloning helps to overcome the problem with transplants of either cells or organs as the immune system recognizes them as foreign. But a patient's body will not reject cells if they are genetically identical to him or her.

The promise of cloning is that it could be used to create stem cells that are essentially the patient's own. An embryo would be cloned from one of the patient's own cells, and destroyed when it was a few days old to produce stem cells. These cells could be chemically guided to become whatever bits of tissue needed replacement – insulin-producing beta-islet cells for diabetics, dopamine-rich neurons for Parkinson's disease, heart tissue. This would be considered as personalized cell therapy.

### *Use of Stem Cells for Drug Testing*

With the ability to isolate, expand and study mesenchymal stem cells (MSCs) in vitro, individual patient's MSCs can be tested for their sensitivity to various drugs. Potential applications are:

- Selection of individual dosing regimens based on the in vitro responsiveness in a simple assay performed using a patient's own MSCs.
- Optimized treatment plans could then be created that efficiently and precisely integrate with the host's expected biological response.
- For example, a patient's sensitivity to a specific dose range of parathyroid hormone (PTH) could be determined in cultures of his MSCs that are induced into the osteogenic lineage pathway.

## Gene Therapy

Gene therapy is defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease state (Jain 2015b). It has three components; (1) identification of the gene mutated in disease and obtaining a healthy copy of that gene; (2) carrier or delivery vehicle called vectors to deliver the healthy gene to a patient's cells; and (3) additional DNA elements that turn on the healthy gene in the right cells and at the right levels. The broad scope of gene therapy includes cells, which may be genetically modified to secrete therapeutic substances such as neurotrophic factors. Ex vivo gene therapy involves the genetic modification of the patient's cells in vitro, mostly by use of viral vectors, prior to reimplanting these cells into the tissues of the patient's body. This is a form of personalized therapy. Another approach to personalizing gene therapy for cancer would be to detect gene groups that are significantly related to a disease by conducting a series of gene expression experiments. Using bioinformatics, gene groups emerging patterns can be analyzed to obtain the most discriminatory genes. The discovered patterns can be used to classify new cells with a higher accuracy than other methods. Based on these patterns, one can consider the feasibility a personalized treatment plan which converts tumor cells into normal cells by modulating the expression levels of a few genes.

### *Stem Cell-Based Personalized Gene Therapy for Cancer*

Gene transfer into human hematopoietic stem cells (HSCs) enables enhancement of anticancer immunity, whereas alteration of HSCs may increase their resistance to cytotoxic drugs. MDR genes can be introduced into HSCs to reduce chemotoxicity and enable the administration of higher doses of chemotherapy. Tumor cell eradication can also be enhanced by genetic modification of chemosensitivity and

immunomodulation. HSCs, genetically engineered with tumor-specific receptors, have been transplanted into mice injected with malignant tumor cells. HSCs produce a variety of immune cells that selectively target and destroy the cancer.

Incorporation of 'omics' data into genetic engineering of stem cells facilitates their use as vectors for delivery of therapeutic genes into specific cancer cells. Thus stem cell-guided gene therapy becomes a promising new frontier in personalized and targeted therapy of cancer (Mavroudi et al. 2014). One risk for therapeutic use of stem cells is their malignant transformation, which can be prevented by appropriate measures.

## Personalized Vaccines

The immunogenetic basis for variations in immune response to vaccines in humans is not well understood. Many factors can contribute to the heterogeneity of vaccine-induced immune responses, including polymorphisms of immune response genes. Identification of genes involved directly or indirectly in the generation of the immune response to vaccines is important. Associations between SNPs in HLA class I and class II genes, cytokine, cell surface receptor, and toll-like receptor genes and variations in immune responses to measles vaccine have been reported (Dhiman et al. 2008). Such information may provide further understanding of genetic variations that influence the generation of protective immune responses to vaccines, and eventually the development of new vaccines. Rapid advances in developing personalized vaccines are already occurring for hepatitis B, influenza, measles, mumps, rubella, anthrax and smallpox vaccines. In addition, newly available data suggest that some vaccine-related adverse events may also be genetically determined and, therefore, predictable.

### *Personalized Cancer Vaccines*

Cancer vaccines attempt to harness the specificity and resistance potentials of the human immune system. The aim of cancer vaccines is to stimulate the immune system to recognize, attack, and destroy tumor cells. In contrast to vaccines for prophylaxis of infectious diseases, cancer vaccines are therapeutic (Jain 2010). These are described in more detail in Chap. 10.

## Antisense Therapy

Antisense molecules are synthetic segments of DNA or RNA, designed to mirror specific mRNA sequences and block protein production. The use of antisense drugs to block abnormal disease-related proteins is referred to as antisense therapeutics.

Synthetic short segments of DNA or RNA are referred to as oligonucleotides. The literal meaning of this word is a polymer made of few nucleotides. Naturally occurring RNA or DNA oligonucleotides may or may not have antisense properties. Antisense therapy is considered to be form of gene therapy because it is modulation of gene function for therapeutic purposes. However, oligonucleotides differ from standard gene therapies because they cannot give rise to proteins but can only block the expression of existing genes. Several antisense approaches use gene therapy technologies, e.g., ribozymes and antisense RNA using vectors.

Emerging clinical evidence supports the notion that antisense oligonucleotides stand a realistic chance of developing into one of the main players of rationally designed anticancer agents. Antisense therapies lend themselves to customization more readily than many other drugs. The reasons are as follows:

- Antisense compounds target a disease at its genetic origin and modulate expression of the gene product whereas conventional pharmaceuticals merely counteract the manifestations of the disease by inhibiting gene products (proteins).
- Antisense compounds can be easily designed and only require information on the nucleic acid sequence encoding a given protein without prior knowledge of the function of that protein.
- Antisense DNA and RNA have an extremely high specificity for their target which cannot be usually achieved by conventional pharmaceuticals.
- Antisense may also provide more disease-specific therapies and have less adverse reactions than conventional pharmaceuticals.

## *RNA Interference*

A refined version of antisense, RNA interference (RNAi), is a cellular mechanism to regulate the expression of genes. RNAi or gene silencing involves the use of a double-stranded RNA (dsRNA), which enters the cell and is processed into short, 21–23 nucleotide dsRNAs termed small interfering RNAs (siRNAs) that are used in a sequence-specific manner to recognize and destroy complementary RNAs (Jain 2015c). RNAi has been shown to control tumor cell growth in vitro. siRNA or plasmids expressing sequences processed to siRNA could provide an exciting new therapeutic modality for treating cancer. Intradigm Corporation (Rockville, MD) is using a siRNA targeting system to modulate the rate of tumor growth and to determine which genes correlate with therapeutic efficiency.

Allele-specific inhibition (ASI) is an approach where cancer cells are attacked at site of loss of heterozygosity (LOH). RNAi approach using oligonucleotide-based drugs may provide the required selectivity for ASI therapeutic approach. siRNAs can not only be used as a tool to study gene function, but might also be used as genotype-specific drugs to mediate ASI. siRNA has been shown to produce genotype-specific inhibition of tumor growth in vivo, by targeting an SNP in POLR2A (gene of the large subunit of RNA polymerase II located in close proximity

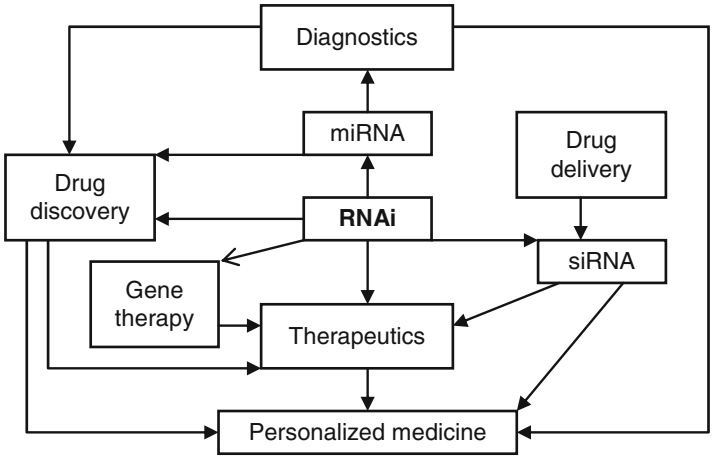


**Fig. 9.1** Role RNAi in development of personalized medicine

to the tumor suppressor gene p53, which frequently shows LOH in cancer cells (Mook et al. 2009). Thus RNAi may play an important role in personalized medicine. A few siRNAs are already in clinical trials. Role of RNAi in the development of personalized medicine is shown in Fig. 9.1.

## *MicroRNAs*

MicroRNAs (miRNAs), small and mostly non-coding RNA gene products, are molecules derived from larger segments of "precursor" RNA that are found in all diverse multicellular organisms. miRNAs are 21–25 nucleotide transcripts that repress gene function through interactions with target mRNAs. Polymorphisms in the miRNA pathway are emerging as a powerful tool to study the biology of a disease and have a potential to be used in disease prognosis and diagnosis. Detection of MiR-polymorphisms holds promise in the field of miRNA pharmacogenomics, molecular epidemiology and for individualized medicine. MiRNA pharmacogenomics can be defined as the study of miRNAs and polymorphisms affecting miRNA function in order to predict drug behavior and to improve drug efficiency. Advancements in the miRNA field indicate the clear involvement of miRNAs and genetic variations in the miRNA pathway in the progression and prognosis of diseases such as cancer, hypertension, cardiovascular disease, and muscular hypertrophy. Various algorithms are available to predict miRNA-target mRNA sites are available. Polymorphisms that may potentially affect miRNA-mediated regulation of the cell can be not only present in the 3′UTR of a miRNA target gene, but also in the genes involved in miRNA biogenesis and miRNA sequences. A polymorphism in processed miRNAs may affect expression of several genes and have serious consequences.

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# Chapter 10
# Personalized Therapy of Cancer

## Introduction

Management of cancer has been unsatisfactory in the past but an understanding of the molecular, genetic and genomic aspects of cancer is accelerating progress in cancer therapy (Jain 2014). Several comprehensive studies have demonstrated the utility of gene expression profiles for the classification of tumors into clinically relevant subtypes and the prediction of clinical outcomes. Role of oncoproteomics in personalized management of cancer was first emphasized in 2004 (Jain 2004). Considerable progress has been made in this field during the past few years. Other factors that drive the development of personalized therapy for cancer are listed in Table 10.1. The preceding chapter described how cancer cell therapy and cancer vaccines can be personalized. Information presented in this section will show personalization of other cancer therapies.

### *Challenges of Cancer Classification*

Cancer is a very heterogeneous disease. Current classifications of cancer are based on the type of tissue of origin, histological appearance and tendency to metastasize. These provide only a limited view of cancer. It is now known that cancer varies both genetically and phenotypically between patients who may have the identical type and stage of cancer. Each person's cancer is as unique as his or her fingerprint. This variability helps to explain unpredictable responses to existing drug therapies that have been observed to date. Large-scale expression monitoring on microarrays has provided the ability to look at cancer at a molecular level and transcription of mRNA messages from genes – transcriptional profiling.

Tumor heterogeneity is underestimated as it is not heterogeneity between tumors, but heterogeneity within an individual tumor as well, which has been mapped out

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**Table 10.1** Factors that drive the development of personalized therapy in cancer

| |
|---|
| Advances in application of proteomic technologies in cancer |
| Advances in cancer vaccine technologies |
| Cancer biomarkers can be used for diagnosis as well as drug targets |
| Examples of personalized treatment of cancer are already in practice |
| Incentive to development from motivated physicians, patients and third party payers |
| Increasing cancer burden with aging US population is a driving force for development. At current incidence rates, the total number of cancer cases is expected to double by 2050 (1.3–2.6 million) |
| Molecular diagnosis of cancer is advancing rapidly |
| Progress in pathophysiology of cancer |
| Search for better treatments due to limited efficacy and toxicity of chemotherapy |
| Sequencing is increasingly applied to understanding cancer and molecular diagnosis |
| Transcriptional profiling in cancer |



(Gerlinger et al. 2012). Multiple samples from each patient's primary and metastatic tumor sites were obtained in a study of renal-cell cancer before and after treatment. About two thirds of the mutations that were found in single biopsies were not uniformly detectable throughout all the sampled regions of the same patient's tumor. A "favorable prognosis" gene profile and an "unfavorable prognosis" gene profile were expressed in different regions of the same tumor. Therefore, a single tumor biopsy cannot be considered representative of the landscape of genomic abnormalities in a tumor. Another finding of this is that different regions of the tumor have different mutations in the very same genes (so-called convergent evolution), including in SETD2, PTEN, and KDM5C, which underscores the importance of changing particular tumor-cell functions as the tumor expands and evolves. From the function of the genes that were targeted for different mutations, it would appear that alterations in epigenetic mechanisms and signal transduction as the tumor evolves are keys to the tumor's survival. Genes that are affected by convergent evolution may be suitable targets for functional inhibition or restoration.

## *Systems Biology of Cancer*

Cancer systems biology addresses the increasing challenge of cancer as a complex, multifactorial disease by using model-based approaches that range from genome-wide regulatory and signaling networks to kinetic models of key pathways. It aims at a holistic view of cancer by use of "omics" technologies and integrates several aspects of cancer including genetics, epigenetics, histology, clinical manifestations and epidemiology. Use of patient-specific computational and mathematical models of cancer will significantly improve the specificity and efficacy of targeted therapy, and will facilitate the development of personalized management of cancer (Du and Elemento 2014). Models of systems biology of cancer and tools for checking them

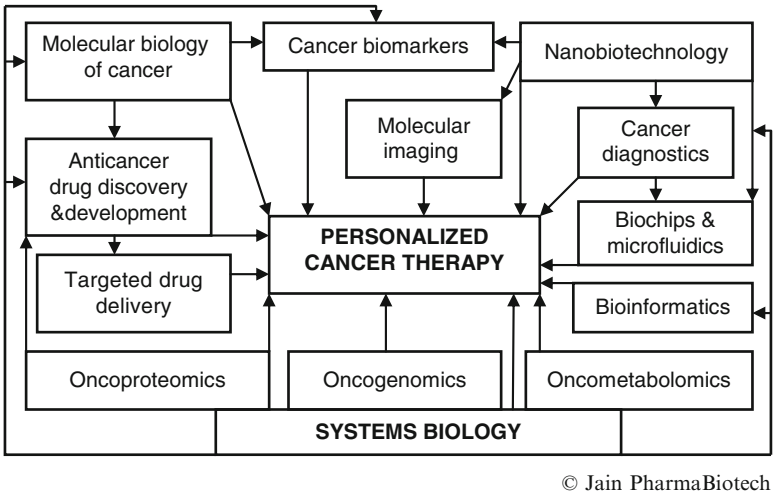


**Fig. 10.1** Relationships of technologies for personalized management of cancer

are reviewed elsewhere (Korsunsky et al. 2014). The authors have pointed out the need for ways to simulate and analyze cancer models efficiently as well as of means to personalize complex heterogeneous model in order to devise the most effective therapy for an individual patient.

## *Relationships of Technologies for Personalized Management of Cancer*

Cancer is a good example of integration of various technologies for personalized management as shown in Fig. 10.1.

The biggest challenge for optimal treatment outcomes in cancer patients is the complex nature of the disease due to cellular heterogeneity and dysfunction of numerous molecular networks as results of genetic as well as environmental disturbances. Systems biology, with its holistic approach to understanding fundamental principles in biology, and the empowering technologies in genomics, proteomics, single-cell analysis, microfluidics, and computational strategies, enables a comprehensive approach to cancer with attempt to unveil the pathogenic mechanisms of diseases, identify disease biomarkers and provide new strategies for drug target discovery. Integration of multidimensional high throughput "omics" measurements from tumor tissues and corresponding blood specimens, together with new systems strategies for diagnostics, enables the identification of cancer biomarkers that can enable presymptomatic diagnosis, stratification of disease, assessment of disease progression, evaluation of patient response to therapy, and the identification of recurrences. Although some aspects of systems medicine are being adopted in

clinical oncology practice through companion molecular diagnostics for personalized therapy, the increasing amount of global quantitative data from both healthy and diseased states is shaping up a transformational paradigm in medicine that is termed 'predictive', 'preventive', 'personalized', and 'participatory' (P4) medicine, which requires new scientific and organizational strategies to translate this approach to the healthcare system (Tian et al. 2012).

## Impact of Molecular Diagnostics on the Management of Cancer

Molecular diagnostics influences cancer management in several ways that lend to personalization (Table 10.2). These technologies are enabling the classification of cancer based on molecular profiles as a basis for more effective personalized therapies. Various tests have been used to predict response to treatment and prognosis.

**Table 10.2** Impact of molecular diagnostics on the management of cancer

| |
|---|
| **Classification of cancer** |
| Analysis of RNA splicing events in cancer |
| Cancer classification using microarrays |
| Cancer stratification based on methylation markers |
| Characteristic of circulating cancer cells |
| eTag assay system for cancer biomarkers |
| Gene expression profiling |
| **Risk assessment and prognosis** |
| Cancer prognosis |
| Detection of mutations for risk assessment and prevention |
| **Prediction of response to treatment** |
| Biopsy testing of tumors for chemotherapy sensitivity |
| Genomic analysis of tumor biopsies to predict response to treatment |
| Prediction of response to radiation therapy |
| Serum nucleosomes as indicators of sensitivity to chemotherapy |
| Testing microsatellite-instability for response to chemotherapy |
| **Diagnostics as guide to therapeutics** |
| Diagnostics for detection of minimal residual disease |
| Detection of resistance to chemotherapy |
| Molecular diagnostics combined with cancer therapeutics |
| **Drug discovery and development** |
| Design of future cancer therapies |
| Screening for personalized anticancer drugs |
| Pharmacogenomic tests for stratification of clinical trials |

### *A Universal NGS-Based Oncology Test System*

In partnership with pharma companies Illumina is developing a universal NGS-based oncology test system for multi-analyte companion diagnostics, which will enable transition from use of single-analyte to panel-based assays for selecting cancer therapies. The new system, which will run assays that detect several variants in parallel, will be used as part of the partners' clinical trials of targeted cancer therapies. Illumina plans to develop, commercialize, and gain regulatory approval for multigene panels for therapy selection, which it previously referred to as Onco Panels. MiSeqDx, the only NGS-based system that has been FDA-cleared to date, will be used to develop the universal test system. Illumina's technology will inform physicians about the molecular make-up of their patients' tumors, enabling them to match medicines to the drivers of disease for personalized management.

### *Analysis of RNA Splicing Events in Cancer*

Alternative splicing has a role in several aspects of cancer treatment, including the failure of the patient to activate the administered drug, high toxicity owing to inappropriate metabolism and variability of the apoptotic thresholds necessary to trigger cell death. Genetic variations within both the patient and the tumor cause changes in the apoptotic threshold and thus differences in both the toxicity and efficacy of a chemotherapy drug. Differential expression of a large number of apoptotic alternative RNA splice variants has been documented in tumors and shows a correlation with drug response. An antisense approach can be used to target specific anti-apoptotic splice variants to lower the apoptotic threshold of a tumor cell and therefore increase the efficacy of chemotherapy drugs. As RNA splicing is deregulated in human cancers, it is likely that such alterations will provide pharmacogenomically relevant markers. Gene expression profiling technologies such as DATA (differential analysis of transcripts with alternative splicing) could be applied to identify RNA splicing differences between tumor biopsies that respond to treatment compared with those that do not respond.

### *Analysis of Chromosomal Alterations in Cancer Cells*

Cancer cells have a remarkable ability to disable some genes and overuse others, allowing their unchecked growth into tumors. The most aggressive of these distortions occurs when cells delete or multiply chunks of their own chromosomes. Cells can simply snip strings of genes from the chromosome, or make many extra copies of the string and reinsert it into the chromosome. A fast and reliable method can identify alterations to chromosomes that occur when cells become malignant (Myers et al. 2004). Genomics tools are used to identify thousands of genes at once and

show how actively they are being used. The data are analyzed by advanced statistical techniques to accurately detect deletions and additions. Many previously unknown additions and deletions have been found in human breast cancer cells by this method. The technique helps to show how cells modify their own genetic makeup and may allow cancer treatments to be tailored more precisely to a patient's disease.

## *Cancer Classification Using Microarrays*

Classification of a cancer based on gene expression profile is important for personalizing cancer therapy. In the process of expression profiling, robotically printed DNA microarrays are used to measure the expression of tens of thousands of genes at a time; this creates a molecular profile of the RNA in a tumor sample. A variety of analytic techniques are used to classify cancers on the basis of their gene-expression profiles. Pattern-recognition algorithms can be used to identify subgroups of tumors that have related gene-expression profiles. Statistical methods are used to relate gene-expression data and clinical data. Determination of tumor marker genes from gene expression data requires bioinformatic tools because expression levels of many genes are not measurably affected by carcinogenic changes in the cells. These molecular markers give valuable additional information for tumor diagnosis/prognosis and will be important for the development of personalized therapy of cancer.

An example of the application of microarrays for gene expression is bladder cancer, a common malignant disease characterized by frequent recurrences. The stage of disease at diagnosis and the presence of surrounding carcinoma in situ are important in determining the disease course of an affected individual. Clinically relevant subclasses of bladder carcinoma have been identified using expression microarray analysis of well-characterized bladder tumors. Gene biomarker panels provide new predictive information on disease progression in tumors compared with conventional staging. Furthermore, gene expression profiles characterizing each stage and subtype identify their biological properties, producing new potential targets for therapy.

Global gene expression analysis using microarrays has been used to characterize the molecular profile of breast tumors. Gene expression variability at the mRNA level can be caused by a number of different events, including novel signaling, downstream activation of transcription enhancers or silencers, somatic mutation, and genetic amplification or deletion. The tyrosine kinase-type cell surface receptor, ERBB2, is an oncogene located on chromosome 17q21.1 that is amplified in 10–40 % of breast tumors. Phenylethanolamine N-methyltransferase (PNMT) is coexpressed with ERBB2 in breast cancer biopsies and also mapped within the same chromosomal location as the ERBB2 gene. Gene amplification of ERBB2 and PNMT is significantly correlated with increased mRNA gene expression.

These results suggest that gene expression profiling of breast biopsies may become a valuable method for adequately characterizing and choosing treatment modality for patients with breast cancer.

Gene expression microarray technology is helpful in all phases of the discovery, development and subsequent use of new cancer therapeutics, e.g., the identification of potential targets for molecular therapeutics. It can be used to identify molecular biomarkers for proof of concept studies, pharmacodynamic endpoints and prognostic markers for predicting outcome and patient selection. Expression profiling can be used alongside gene knockout or knockdown methods such as RNA interference.

## *Catalog of Cancer Genes for Personalized Therapy*

Personalized medicine for cancer will eventually require a comprehensive catalog of cancer genes to enable physicians to select the best combination therapy for each patient based on the cellular pathways disrupted in their tumor and the specific nature of the disruptions. Such a catalog will also guide therapeutic development by identifying druggable targets.

Although a few cancer genes are mutated in a high proportion of tumors of a given type (>20 %), most are mutated at intermediate frequencies (2–20 %). To explore the feasibility of creating a comprehensive catalog of cancer genes, researchers at Broad Institute (Cambridge, MA) analyzed somatic point mutations in exome sequences from ~5,000 human cancers and their matched normal-tissue samples across 21 cancer types (Lawrence et al. 2014). Using the MutSig tool, which weighs mutational burden as compared to the background mutation rate, mutational clustering, and enrichment of mutations in conserved regions, the researchers searched for candidate cancer genes. After filtering the data, they found >3 million SNVs, among other mutations, which are almost all known cancer genes in these tumor types. Their analysis also identified 33 genes that were not previously known to be significantly mutated in cancer, including genes related to proliferation, apoptosis, genome stability, chromatin regulation, immune evasion, RNA processing and protein homeostasis.

By combining the 22 MutSig lists, the researchers developed Cancer5000 set of 254 genes. Down-sampling analysis indicates that larger sample sizes will reveal many more genes mutated at clinically important frequencies. Many new candidate cancer genes remain to be discovered beyond those in the current Cancer5000 set. Researchers estimate that near-saturation may be achieved with 600–5,000 samples per tumor type, depending on background mutation frequency. The results may help to guide the next stage of cancer genomics. A comprehensive cancer catalog, would not only guide personalized cancer treatment, but also improve our understanding of the mechanisms at play in cancer as well as spur the development of new therapies.

## *Circulating Cancer Cell Analysis for Personalizing Therapy*

Blood samples can now analyzed for circulating tumor cells (CTCs) by nucleic acid methods to isolate tumor-associated or tumor-specific mRNA. Detection of extremely low concentrations of rare CTCs in the blood is still a challenge. IsoFlux System (Fluxion Biosciences) utilizes a unique microfluidic design to provide automated cell introduction, trapping, sealing, whole-cell formation and recording protocols. IsoFlux System is being used to isolate, recover, and analyze rare CTCs to provide a real-time "liquid biopsy" of samples for molecular analysis. The focus is on breast and lung cancers with the goal of subtyping different forms of the disease and developing treatments personalized to each individual patient.

### Detection of Loss of Heterozygosity

Many cancers are characterized by chromosomal aberrations that may be predictive of disease outcome. Human neuroblastomas are characterized by loss of heterozygosity (LOH), the deletion of one copy of a pair of genes at multiple chromosomal loci. When the gene involved is a tumor suppressor gene, LOH removes a brake on uncontrolled cell growth, the growth that is the hallmark of cancer. A customized gene chip has been developed to assess region-specific LOH by genotyping multiple SNPs simultaneously in DNA from tumor tissues (Maris et al. 2005). Unlike gene expression microarrays, which detect varying levels of RNA to measure the activity levels of different genes as DNA transfers information to RNA, the current microarray directly identifies changes in DNA. Rather than covering the entire genome, the microarray focuses on suspect regions of chromosomes for signs of deleted genetic material known to play a role in the cancer. Detection of LOH in this assay may not require comparison to matched normal DNAs because of the redundancy of informative SNPs in each region. This customized tag-array system for LOH detection is rapid, results in parallel assessment of multiple genomic alterations, and may speed identification of and/or assaying prognostically relevant DNA copy number alterations in many human cancers. Identifying the correct risk level allows doctors to treat aggressive cancers appropriately, while not subjecting children with low-risk cancer to overtreatment.

### BEAMing Technology for Analysis of Circulating Tumor DNA

Inostics' BEAMing (Beads, Emulsions, Amplification, and Magnetics) – a digital technology that combines emulsion based digital PCR with magnetic beads and flow cytometry – enables quantification of mutant DNA. It is a non-invasive, highly-sensitive, real-time "liquid biopsy" for screening, prognosis, prediction, and monitoring of cancer. Key features are:

- Starting material: plasma, serum, or tissue (FFPE/frozen)
- Starting DNA amount: ≥1 genome equivalent (6.6 pg human genomic DNA)

- Detection capability: 0.01 % (mutant/total DNA) 1–4 (i.e. ≥1 mutant DNA molecule in 10,000 wild type DNA molecules)
- Turnaround time 2–4 days
- Available for analysis of 130 different mutations in >15 different cancer genes

## *Diagnosis of Cancer of an Unknown Primary*

Metastatic cancer of unknown primary site (CUP) accounts for ~3 % of all malignant neoplasms and is therefore one of the 10 most frequent cancer diagnoses in humans. Patients with CUP present with metastatic disease for which the site of origin cannot be identified at the time of diagnosis. It is now accepted that CUP represents a heterogeneous group of malignancies that share a unique clinical behavior and, presumably, unique biology. Extensive work-up with specific pathology investigations (immunohistochemistry, electron microscopy, molecular diagnosis) and modern imaging technology (CT, mammography, PET scan) have resulted in some improvements in diagnosis, but the primary site remains unknown in most patients. The most frequently detected primaries are carcinomas hidden in the lung or pancreas. Several favorable sub-sets of CUP have been identified, which are responsive to systemic chemotherapy and/or locoregional treatment. Identification and treatment of these patients is important. The considered responsive sub-sets to platinum-based chemotherapy are the poorly differentiated carcinomas involving the mediastinal-retroperitoneal nodes, the peritoneal papillary serous adenocarcinomatosis in females and the poorly differentiated neuroendocrine carcinomas. Other tumors successfully managed by locoregional treatment with surgery and/or irradiation are the metastatic adenocarcinoma of isolated axillary nodes, metastatic squamous cell carcinoma of cervical nodes, or any other single metastatic site. Diagnosis of CUP is important for personalized management of cancer. Diagnostics are being developed for CUP using microarrays and gene expression analysis.

## *Diagnostics for Detection of Minimal Residual Disease*

In the pre-molecular diagnostic era, hematologists used the microscope to identify a complete remission of leukemia after treatment with chemotherapy. In a hematologic complete remission, it is known that a large portion of the leukemic cells remain out of sight. These cells, invisible to the microscopist, are the components of an important clinical problem termed “minimal residual disease (MRD)”. RT-PCR has been used to detect BCR-ABL transcripts in chronic myeloid leukemia (CML) in the chronic phase. BCR-ABL transcripts were measured in blood samples of patients in remission following treatment with imatinib using RT-PCR with BCR as a control gene, thus ensuring standardization of the method in the participating laboratories. There is a progressive reduction of the leukemic mass that exists below

the level of cytogenetic visibility, a decrease that is still ongoing in many patients 15 months after attainment of a complete cytogenetic remission with imatinib treatment. Moreover, patients who attained such a molecularly defined minimal tumor burden had a higher rate of progression-free survival than those who did not. The molecular data thus provide support for the position of imatinib as the drug of choice in CML.

## *DNA Repair Biomarkers*

Most chemotherapeutics and radiation therapy work by damaging DNA. All solid cancers have multiple pathways and some of these can enable tumor cells to survive DNA damage induced by chemotherapeutic treatments. Therefore, inhibitors of specific DNA repair pathways might prove effective when used in combination with DNA-damaging chemotherapeutic drugs. In addition, alterations in DNA repair pathways that arise during tumor development can make some cancer cells reliant on a reduced set of DNA repair pathways for survival. There is evidence that drugs that inhibit one of these pathways in such tumors could prove useful as single-agent therapies, with the potential advantage that this approach could be selective for tumor cells and have fewer side effects (Helleday et al. 2008). Proteomic biomarkers in each of the pathways differ according to the type of cancer but they overlap. Since most available cancer therapeutics work by inducing DNA damage, which causes cell death, monitoring specific DNA repair biomarkers may indicate whether the therapeutic is producing tumor cell death. DNA repair biomarkers could enable physicians to monitor treatment effectiveness from solid tumor samples.

## *Fluorescent In Situ Hybridization*

Fluorescence in situ hybridization (FISH) is now used routinely in the clinical laboratory during all phases of management of a number of malignancies. The specific associations between distinct chromosomal abnormalities and different types of cancers will necessitate simultaneous detection of multiple abnormalities using multicolor/multiplex FISH tests more often in the near future and will bring the concept of personalized medicine in cancer closer to reality than ever before.

## *Gene Expression Profiling*

Aberrant expression of p53, myc, and ras is known to initiate tumorigenesis and progression of tumors. p53 mutations are associated with drug resistance and treatment failure while activation of oncogenes c-myc and ras is often associated with

elevated cell proliferation. Expression profiling of these genes can provide useful information about cancer and planning its personalized treatment.

Microarray methods have revealed unexpected subgroups within the diagnostic categories of the hematologic cancers that are based on morphology and have demonstrated that the response to therapy is dictated by multiple independent biologic features of a tumor. Some examples of applications of this approach are:

- These expression signatures can be combined to form a multivariate predictor of survival after chemotherapy for diffuse large-B-cell lymphoma.
- Gene-expression profiling has been used as an alternative approach to mapping chromosomal translocations in leukemias. Gene-expression signatures can be combined with the use of statistical algorithms to predict chromosomal abnormalities with a high degree of accuracy.
- In B-cell acute lymphoblastic leukemia, gene-expression profiling at the time of diagnosis provides information that could predict which patients would relapse and which would remain in continuous complete remission.
- ZAP-70 gene expression identifies a chronic lymphocytic leukemia (CLL) subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. RT-PCR and immunohistochemical assays for ZAP-70 expression can be applied clinically and would yield important prognostic information for CLL patients.

An important goal is to develop a platform for routine clinical diagnosis that can quantitatively measure the expression of a few hundred genes. Such a diagnostic platform would enable a quick determination of important molecular subgroups within each hematologic cancer. As new clinical trials designed, one must include genomic-scale gene-expression profiling in order to identify the genes that influence the response to the agents under investigation. Thus the molecular diagnosis of the hematologic cancers can be refined on the basis of new advances in treatment and facilitate the development of tailored therapies for molecularly defined diseases.

Gene expression profiling has been done of prostate tumors using IHC on tissue microarrays. Positive staining for MUC1, a gene highly expressed in the subgroups with aggressive clinicopathological features, is associated with an elevated risk of recurrence, whereas strong staining for AZGP1, a gene highly expressed in the other subgroup, is associated with a decreased risk of recurrence. In multivariate analysis, MUC1 and AZGP1 staining are strong predictors of tumor recurrence independent of tumor grade, stage, and preoperative prostate-specific antigen (PSA) levels. These findings suggest that prostate tumors can be usefully classified according to their gene expression patterns, and these tumor subtypes may provide a basis for improved stratification for prognosis and treatment.

Gene-expression profiling has been used to improve the design of cancer drugs that have shown some promise in clinical trials. Some of the cancer signatures can predict clinical response in individuals treated with anticancer drugs. Notably, signatures developed to predict response to individual agents, when combined, could also predict response to multidrug regimens. Finally, integration of chemotherapy response signatures with signatures of oncogenic pathway deregulation may help to

identify new therapeutic strategies that make use of all available drugs. The development of gene expression profiles that can predict response to commonly used cytotoxic agents provides opportunities to better use these drugs, including their use in combination with existing targeted therapies.

### OnkoMatch Tumor Genotyping

OnkoMatch™ tumor genotyping (GenPath Oncology), based on PCR amplification followed by single base extension detection of hotspot mutations that have been identified as key driver mutations, provides a reliable and robust tumor genotyping platform for detecting 68 mutations (including EGFR, BRAF and KRAS) across 14 oncogenes. It was developed at the Massachusetts General Hospital where SNaPshot Multiplex System has been applied for genotyping tumors such as NSCLC and in influencing treatment decisions as well as directing patients toward relevant clinical trials (Sequist et al. 2011).

### Gene Expression Profiles Predict Chromosomal Instability in Tumors

Microscopic examination of tumor specimens cannot always predict a cancer's aggressiveness, leading to increased interest in molecular approaches to diagnosis. A genetic profile indicating chromosomal instability – an increased tendency to develop chromosomal aberrations that are critical in cancer development – is predictive of clinical outcome in a broad range of cancer types. Chromosomal instability leads to a condition known as aneuploidy, in which chunks of DNA are either missing or duplicated. Abnormal expression levels of genes at the different chromosomal locations indirectly reflect the degree of aneuploidy and thus the degree of chromosomal instability.

A 25-gene signature of chromosomal instability has been identified from specific genes whose expression was consistently correlated with total functional aneuploidy in several cancer types (Carter et al. 2006). This signature was a significant predictor of clinical outcomes in a variety of cancers (breast, lung, medulloblastoma, glioma, mesothelioma and lymphoma). It could also differentiate between primary tumors and tumor metastases, and in grade 1 and grade 2 breast cancers, distinguished the more aggressive cancer within each grade. Using gene expression data from 18 previous studies of cancer, representing 6 cancer types, the authors found that this genetic profile, or signature, predicted poor clinical outcome in 12 of the populations studied. The technique may form the basis of a diagnostic tool that could be used in the clinic and also help in the search for cancer drugs that reduce chromosomal instability. This approach would be useful for developing personalized therapy of cancer.

## *Isolation and Characterization of Circulating Cancer Cells*

Viable tumor-derived epithelial cells (circulating cancer cells or CTCs) have been identified in peripheral blood from cancer patients and are probably the origin of intractable metastatic disease. Although extremely rare, CTCs represent a potential alternative to invasive biopsies as a source of tumor tissue for the detection, characterization and monitoring of non-hematologic cancers. The ability to identify, isolate, propagate and molecularly characterize CTC subpopulations could further the discovery of cancer stem cell biomarkers and expand the understanding of biology of the metastatic process.

Current strategies for isolating CTCs are limited to complex analytic approaches that generate very low yield and purity. A unique microfluidic platform (the 'CTC-chip') is capable of efficient and selective separation of viable CTCs from peripheral whole blood samples, mediated by the interaction of target CTCs with antibody (EpCAM)-coated microposts under precisely controlled laminar flow conditions, and without requisite pre-labeling or processing of samples (Nagrath et al. 2007). The CTC-chip has successfully identified CTCs in the peripheral blood of patients with metastatic lung, prostate, pancreatic, breast and colon cancer in 99 % of samples. Given the high sensitivity and specificity of the CTC-chip, its potential utility was tested in monitoring response to anticancer therapy. In a small cohort of patients with metastatic cancer undergoing systemic treatment, temporal changes in CTC numbers correlated reasonably well with the clinical course of disease as measured by standard radiographic methods. Thus, the CTC-chip provides a new and effective tool for accurate identification and measurement of CTCs in patients with cancer. It has broad implications in advancing both cancer biology research and clinical cancer management, including the detection, diagnosis and monitoring of cancer (Sequist et al. 2009). CTC-Chip has been applied for the personalized management of NSCLC (see under lung cancer).

## *Modulation of CYP450 Activity for Cancer Therapy*

Metabolism mediated by cytochrome P450 isoenzymes is known to play a major part in the biotransformation of anticancer agents in vivo. Variability between individuals in the pharmacokinetics of anticancer chemotherapeutic agents has an impact on therapeutic efficacy and safety. Since most anticancer agents are transformed by enzymes, a better knowledge of the biotransformation pathways of cyclophosphamide, ifosfamide, tamoxifen, docetaxel, paclitaxel, and irinotecan could help improve treatment outcome. Furthermore, a better understanding of the metabolism of anticancer agents through phenotyping and genotyping approaches will facilitate the prediction of interactions between drugs. More clinical evidence is needed on the metabolic transformation and drug interactions with these agents to improve cancer therapeutics.

## *NanoFlares for Detection of CTCs*

NanoFlares are nanoconstructs that enable live-cell detection of intracellular mRNA. NanoFlares, when coupled with flow cytometry, can be used to fluorescently detect genetic markers of CTCs in the context of whole blood enabling detection of as few as 100 live cancer cells per mL of blood and subsequent culture of those cells (Halo et al. 2014). This technique can also be used to detect CTCs in a murine model of metastatic breast cancer. As such, NanoFlares are the first genetic-based approach for detecting, isolating, and characterizing live cancer cells from blood and may provide new opportunities for cancer diagnosis, prognosis, and personalized therapy.

## *Pathway-Based Analysis of Cancer*

### Conversion of Gene-Level Information into Pathway-Level Information

Gene-level information obtained by gene expression studies needs to be converted into pathway-level level information to generate biologically relevant representation of each tumor sample. An algorithm, Pathifier, infers pathway deregulation scores for each tumor sample on the basis of expression data in a context-specific manner for every particular dataset and type of cancer that is being investigated (Drier et al. 2013). Multiple pathway-based representation of algorithm on three colorectal cancer (CRC) datasets as well as two glioblastoma multiforme (GBM) datasets was shown to be reproducible, preserved much of the original information, and enabled inference of complex biologically significant information. They discovered several pathways that were significantly associated with survival of GBM patients and two whose scores are predictive of survival in CRC: CXCR3-mediated signaling and oxidative phosphorylation. They also identified a subclass of proneural and neural GBM with significantly better survival, and an EGF receptor-deregulated subclass of CRC. Pathifier is useful for personalized management of cancer.

### Personalized Therapies Based on Oncogenic Pathways Signatures

The ability to define cancer subtypes, recurrence of disease and response to specific therapies using DNA microarray-based gene expression signatures has been demonstrated in several studies. By introducing a series of oncogenes into otherwise normal cells and comparing gene expression patterns in normal cells versus cells harboring oncogenes, it can be shown that each cellular signaling pathway is associated with a unique gene expression signature. When evaluated in several large collections of human cancers, these gene expression signatures identify patterns of pathway deregulation in tumors and clinically relevant associations with disease

outcomes. Combining signature-based predictions across several pathways identifies coordinated patterns of pathway deregulation that distinguish between specific cancers and tumor subtypes. The majority of adenocarcinomas of the lung are found to be deregulated for the oncogene Ras, while only a tiny minority of squamous cell carcinomas exhibited Ras deregulation. Hence, deregulation of the Ras pathway is an important signature of adenocarcinomas but not of squamous cell carcinoma.

Clustering tumors based on pathway signatures further defines prognosis in respective patient subsets, demonstrating that patterns of oncogenic pathway deregulation underlie the development of the oncogenic phenotype and reflect the biology and outcome of specific cancers. Predictions of pathway deregulation in cancer cell lines are also shown to predict the sensitivity to therapeutic agents that target components of the pathway. Linking pathway deregulation with sensitivity to therapeutics that target components of the pathway provides an opportunity to make use of these oncogenic pathway signatures to guide the use of personalized cancer therapies. If the Ras and Myc pathways are activated in a tumor, physicians could choose drugs that target only Myc and Ras. If the SRC and E2F3 pathways are highly active, then drugs can be selected that target these pathways. Because tumors arise from multiple defective genes and their malfunctioning proteins, treatments must target multiple genes and their pathways. The likelihood that someone will be cured by a single drug is low, and the new approach can guide physicians as to which combination of drugs will most likely produce the best outcome.

The next step in the research is to validate the new method in samples from cancer patients who have been treated with one of the pathway-specific drugs to determine if the pathway predictors are able to select those patients most likely to respond to the drug. A positive result would then form the basis for a clinical study that would evaluate the effectiveness of the pathway prediction to guide the most effective use of therapeutics.

## *Quantum Dot-Based Test for DNA Methylation*

DNA methylation contributes to carcinogenesis by silencing key tumor suppressor genes. An ultrasensitive and reliable quantum dot (QD)-based assay, MS-qFRET (fluorescence resonance energy transfer), can detect and quantify DNA methylation (Bailey et al. 2009). The direct application of MS-qFRET on clinical samples offers great promise for its translational use in early cancer diagnosis, and prognostic assessment of tumor behavior, as well as monitoring response to therapeutic agents. Gene DNA methylation indicates a higher risk of developing cancer and is also seen as a warning sign of genetic mutations that lead to development of cancer. Moreover, since different cancer types possess different genetic biomarkers, e.g. lung cancer biomarkers differ from leukemia biomarkers, the test should identify the cancer a patient is at risk of developing. This test could be used for frequent screening for cancer and replacing traditionally invasive methods with a simple blood test. It could also help determine whether a cancer treatment is effective and thus enable personalized chemotherapy.

## *Role of Molecular Imaging in Personalized Therapy of Cancer*

In oncology, if cancer cells are removed from their microenvironment, their pattern of gene expression changes because the behavior of tumor cells is inextricably linked to their environments. Therefore, noninvasive, quantitative means of detecting gene and protein activity are essential. In vivo imaging is one method for achieving this. Various technologies available for this purpose are PET scanning, SPECT and MRI. Ultrasound and CT are being re-engineered to reflect information at the cellular level. In vivo optical imaging technologies have matured to the point where they are indispensable laboratory tools for small animal imaging. Human applications are being explored and the future for clinical optical imaging techniques looks bright. Merging these molecular imaging techniques with minimally or noninvasive image-guided therapeutic delivery techniques is an important goal in the fight against cancer.

In investigational and clinical oncology there is a need for imaging technologies that will indicate response to therapy prior to clinical evidence of response. The conventional imaging methods such as CT and MRI enable anatomic measurements of the tumor. This may be useful for assessing response to traditional cytotoxic agents where tumor shrinkage occurs early. In contrast to this, molecularly targeted agents tend to induce arrest of cancer cell growth and development, but not necessarily significant tumor shrinkage in the short term. Thus there is a need for functional or molecular imaging methods that would give information about what is happening in the tumor at the molecular level. One example of this approach is an attempt to find an explanation for poor performance of some antiangiogenesis drugs in clinical trials despite abundant preclinical evidence that the drugs should work. Noninvasive molecular imaging is needed to identify patients that are suitable for a particular targeted therapy, and to determine if the drug is reaching its target and in sufficient quantities to block the target. The molecularly targeted approaches enable the therapy to be individually tailored to a given patient's tumor and metabolism.

CT may not be optimally suitable for assessment of oncolytic virus treatments because of paradoxical inflammatory tumor swellings, which result from virus treatments, particularly when viruses are armed with immunostimulatory molecules. In a comparative study of patients treated by viral oncolysis, FDG-PET was more sensitive in detection of responses than tumor size determined by CT (Koski et al. 2013).

### Functional Diffusion MRI

Functional diffusion MRI scan could help physicians decide quickly whether treatment for brain tumors is having any effect. The scan uses MRI to track the Brownian motion of water through the brain (Moffat et al. 2005). Tumor cells block the flow of water, so as those cells die, water diffusion patterns change, and the new MRI technology can track it. Application of this technique in patients with malignant

brain tumors showed changes in the diffusion map if chemotherapy or radiation therapy was having any effect. It worked within 3 weeks, 10 weeks before traditional MRI techniques of assessing whether therapy is working. Usually, patients get 7 weeks of treatment, followed by a traditional MRI scan 6 weeks afterwards to see if the tumor has shrunk. If it does not, the management approach may be altered depending on the tumor. Speeding up this process can save patients from often-uncomfortable treatments that may be a waste of time. Use of MRI tumor diffusion values to accurately predict the treatment response early on could enable some patients to switch to a more beneficial therapy and avoid the side effects of a prolonged and ineffective treatment. There are plans to test the technique with breast cancer as well as head and neck cancer.

### FDG-PET/CT for Personalizing Cancer Treatment

Multimodality imaging, as represented by PET, has a definite role in the evaluation of a patient with cancer. Fluorodeoxyglucose (FDG)-PET is rapidly becoming the key investigative tool for the staging and assessment of cancer recurrence. In the last 5 years, PET has also gained widespread acceptance as a key tool used to demonstrate early response to intervention and therapy, whereas changes in size of tumor as shown by CT alone may take longer. This clinical need is being addressed with FDG-PET/CT, because of its inherent ability to demonstrate (before other biomarkers of response) if disease modification has occurred (Ben-Haim and Ell 2009). This is an important factor in personalizing cancer treatment.

In non-small cell lung cancer (NSCLC), reduction of metabolic activity as demonstrated by FDG-PET after one cycle of chemotherapy is closely correlates with final outcome of therapy. Using metabolic response as an end point may shorten the duration of phase II studies evaluating new cytotoxic drugs and may decrease the morbidity and costs of therapy in non-responding patients. Another example of a generic functional imaging method is the use of FDG-PET to look at the response of gastrointestinal stromal tumor (GIST) to Gleevec. Preliminary studies show marked decrease of FDG uptake in GIST tumors within 24 h in patients who go on to show clinical response to Gleevec. PET accurately diagnosed tumor response in 85 % of patients at 1 month and 100 % at 3–6 months whereas CT was found to be accurate in 44 % of patients at 1 month, 60 % at 3 months, and 57 % at 6 months (Antoch et al. 2004). Radiolabeled annexin V may provide an early indication of the success or failure of anticancer therapy on a patient-by-patient basis as an in vivo marker of tumor cell killing. The temporal patterns of tumor cell loss has been demonstrated by SPECT and provides a better understanding of the timing of radiolabeled annexin V uptake for its development as a biomarker of therapeutic efficacy.

Abnormal tryptophan metabolism catalyzed by indoleamine 2,3-dioxygenase may play a prominent role in tumor immunoresistance in many tumor types, including lung tumors. Prolonged retention of alpha-(11)C-methyl-l-tryptophan (AMT), a PET tracer for tryptophan metabolism, in NSCLCs suggests high metabolic rates of tryptophan in these tumors. AMT PET/CT may be a clinically useful molecular

imaging method for personalized cancer treatment by identifying and monitoring patients who have increased tumor tryptophan metabolism and are potentially sensitive to immunopharmacotherapy with indoleamine 2,3-dioxygenase inhibitors (Juhász et al. 2009).

### Image-Guided Personalized Drug Delivery in Cancer

Image-guided drug delivery (IGDD) in cancer is a form of individualized therapy where imaging methods are used in guidance and monitoring of localized and targeted delivery of therapeutics to the tumor. A systematic approach to IGDD requires mechanisms for targeting, delivery, activation, and monitoring of the process. Although the goal in IGDD is to optimize the therapeutic ratio through personalized image-guided treatments, a major challenge is in overcoming the biological barriers to the delivery of therapeutics into tumors and cells. Physiologic and quantitative imaging techniques may serve as enabling tools that could potentially transform many existing challenges into opportunities for advancement of the field (Tandon and Farahani 2011).

### Tumor Imaging and Elimination by Targeted Gallium Corrole

Sulfonated gallium(III) corroles are intensely fluorescent macrocyclic compounds that spontaneously assemble with carrier proteins to undergo cell entry. In vivo imaging and therapeutic efficacy of a tumor-targeted corrole noncovalently assembled with a heregulin-modified protein directed at the human EGFR. Systemic delivery of this protein-corrole complex results in tumor accumulation, which can be visualized in vivo owing to intensely red corrole fluorescence. Targeted delivery in vivo leads to tumor cell death while normal tissue is spared in contrast with the effects of doxorubicin, which can elicit cardiac damage during therapy and required direct intratumoral injection to yield similar levels of tumor shrinkage compared with the systemically delivered corrole (Agadjanian et al. 2009). The targeted complex ablated tumors at >5 times a lower dose than untargeted systemic doxorubicin, and the corrole does not damage heart tissue. Complexes remain intact in serum and the carrier protein elicits no detectable immunogenicity. The sulfonated gallium(III) corrole functions both for tumor detection and intervention with safety and targeting advantages over standard chemotherapy.

### Future Prospects of Molecular Imaging in Management of Cancer

Molecular imaging can improve therapeutic strategies that provide better patient selection for therapeutic personalization than conventional methods and provides a variety of new tools to accelerate the development of cancer therapies. The recent drive to develop molecular imaging probes and standardize molecular imaging

techniques is creating the scaffolding for the evolving paradigm shift to personalized cancer therapy (Kurdziel et al. 2008). Molecular imaging, also referred to by the term "virtual biopsy" can provide a more complete systematic picture of the living tumor, but it is not likely to replace and would rather be complementary to pathology, IHC and genomic analysis. However, molecular imaging methods are certainly complementary to biopsy. The primary advantages of molecular imaging are that it is nondestructive, non- or minimally invasive and thus easier on patients, permits the collection of data over time thus enabling post therapy evaluations, and provides near real-time functional information, and encompasses large volumes of tissue (the whole body in most case). One drawback of the molecularly targeted approaches is the expensive development and lack of interest in the pharmaceutical industry to combine functional imaging with anticancer drugs in development.

### *Unraveling the Genetic Code of Cancer*

A systematic analysis has been carried out for determining the sequence of well-annotated human protein-coding genes in two common tumor types to identify genetic alterations in breast and colorectal cancers (Sjoblom et al. 2006). Analysis of 13,023 genes in 11 breast and 11 colorectal cancers revealed that individual tumors accumulate an average of ~90 mutant genes but that only a subset of these contribute to the neoplastic process. Using stringent criteria to delineate this subset, the authors identified 189 genes (average of 11 per tumor) that were mutated at significant frequency. The vast majority of these genes were not known to be genetically altered in tumors and are predicted to affect a wide range of cellular functions, including transcription, adhesion, and invasion. These data define the genetic landscape of two human cancer types, provide new targets for diagnostic and therapeutic intervention, and open fertile avenues for basic research in tumor biology. The mutated genes in breast and colon cancers were almost completely distinct, suggesting very different pathways for the development of each of these cancer types. Each individual tumor appeared to have a different genetic blueprint, which could explain why cancers can behave very differently from person to person. The discovery could also lead to better ways to diagnose cancer in its early, most treatable stages, and personalized treatments. Maximizing the numbers of targets available for drug development in a specific cancer means that patients will ultimately receive more personalized, less toxic drugs.

## Cancer Prognosis

Molecular diagnostics provide an easier, less invasive way to determine cancer prognosis. For example, patients with the greatest degree of amplification (in terms of gene copy numbers) of the N-*myc* gene in neuroblastoma, a highly malignant

tumor, have the worst prognosis. Molecular tests for TP53 and RER are already considered to offer prognostic value in certain types of cancer. In addition, the ability to locate residual cancer by molecular methods can aid in predicting the course of the disease.

A more accurate means of prognosis in breast cancer will improve the selection of patients for adjuvant systemic therapy. Gene signatures seem to be promising for predicting outcome, and should pave the way for new therapies that are tailored to the patient. Gene-expression profiles based on microarray analysis can be used to predict patient survival in early-stage lung adenocarcinomas. The identification of a set of genes that predict survival in early-stage lung adenocarcinoma allows delineation of a high-risk group that may benefit from adjuvant therapy. Differentially expressed genes were used to generate a 186-gene "invasiveness" gene signature (IGS), which is strongly associated with metastasis-free survival and overall survival for four different types of tumors: breast cancer, medulloblastoma, lung cancer, and prostate cancer (Liu et al. 2007). The prognostic power of the IGS was increased when combined with the wound-response signature based on transcriptional response of normal fibroblasts to reveal links between wound healing and cancer progression.

## *Detection of Mutations for Risk Assessment and Prevention*

Tests with the greatest potential for risk assessment include those that target mutations in the following genes:

- BRCA1 and BRCA2 (for breast and ovarian cancers);
- MLH1 and MSH2 (colon cancer);
- APC (for familial adenomatous polyposis);
- RET (for medullary thyroid cancer);
- TP53 (for several tumors);
- CDKN2A (for melanoma);
- RB1 (for retinoblastoma).

Detection of mutation in an individual would theoretically lead to increased surveillance. Lifestyle changes might be advised to avoid known risk factors for progression of cancer. In some cases, prophylactic surgery may be recommended. In addition, some chemotherapeutic agents might be prescribed on a preventive basis. Detection of a mutation may be followed by surveillance-oriented examinations, including those involving colonoscopy, mammography, measurement of prostate-specific antigen, and other tests. This tactic will promote the early detection of cancer and early management. Current molecular research is expected to reveal other markers for early diagnosis of cancer. In addition, the possibility of generating genetic profiles for individual tumors offers unique opportunities for distinguishing between metastases and primary tumors.

Effective targeted cancer therapeutic development depends upon distinguishing disease-associated 'driver' mutations, which have causative roles in pathogenesis of malignancy, from 'passenger' mutations, which are dispensable for cancer initiation and maintenance. Translational studies of clinically active targeted therapeutics can definitively discriminate driver from passenger lesions and provide valuable insights into human cancer biology.

## *Hematological Cancer Risk Inferred from Blood DNA Sequence*

Detectable clonal expansions most frequently involved somatic mutations in three genes (DNMT3A, ASXL1, and TET2) have been implicated in hematologic cancers. A study has shown that clonal hematopoiesis is a strong risk factor for subsequent hematologic cancer as ~42 % of hematologic cancers in this cohort arose in persons who had clonality at the time of DNA sampling, >6 months before a first diagnosis of cancer (Genovese et al. 2014). Clonal hematopoiesis with somatic mutations is readily detected by means of DNA sequencing, is increasingly common as people age, and is associated with increased risks of hematologic cancer and death. A subset of the genes that are mutated in patients with myeloid cancers is frequently mutated in apparently healthy persons; these mutations may represent characteristic early events in the development of hematologic cancers.

In the future, it may be possible to refine DNA analysis to develop strategies for early detection and prevention of hematologic cancer. DNA analysis will offer three important capabilities: ascertaining high-risk states, monitoring progression or remission of these states, and follow-up of transforming mutations before clinically apparent illness. The following research directions could bring DNA sequencing for clonal hematopoiesis closer to clinical usefulness:

1. Some somatic mutations are likely to be associated with a particularly high risk of subsequent cancer; larger studies could identify such mutations.
2. Single-cell analyses might identify high-risk combinations of mutations occurring in the same cells.
3. Sequencing of specific cell types might identify mutation and cell-type combinations with higher predictive value.
4. Initial detection of clonal hematopoiesis might justify periodic screening for the presence of cooperating mutations at low allele frequencies that could presage cancer.

Large clinical studies will be needed to evaluate these possibilities. DNA sequencing, by improving our ability to predict future disease in asymptomatic persons, may eventually replace the traditional demarcation between illness and health with a continuum of ascertainable genomic states that are associated with elevated risks of illness.

## Impact of Biomarkers on Management of Cancer

Biomarkers are playing an important role in the diagnosis as well as management of cancer. Some examples are given here.

### *HER-2/neu Oncogene as a Biomarker for Cancer*

HER-2/neu oncogene, also referred to as c-erbB-2, encodes a protein with a molecular weight of 185,000 Da and is structurally related to the human epithelial growth factor receptor. The full length p185 HER-2/neu protein is composed of a cytoplasmic domain with tyrosine kinase activity, a transmembrane domain and an extracellular domain (ECD) that is shed from the surface of breast cancer cells. Numerous studies have shown that the shed ECD of HER-2/neu is a glycoprotein with a molecular weight between 97 and 115 kDa and designated p105. The ECD can be accurately quantified in serum with an ELISA that uses MAbs directed to the external epitopes of the HER-2/neu protein. Many publications show that the ECD is shed into the blood of normal individuals and can be elevated in women with metastatic breast cancer. Many of these serum HER-2/neu studies have confirmed the substantial data from tissue studies that HER-2/neu is a biomarker of poor prognosis, shorter overall survival and biological aggressiveness. Scientific studies suggest that quantitation of the ECD may have several important clinical applications such as monitoring breast cancer patients with metastatic disease.

Various reports have shown that 30–50 % of women with positive HER-2/neu tumors at primary diagnosis develop elevated levels of serum HER-2/neu with progression to metastatic breast cancer. These studies have also illustrated that monitoring serum ECD levels post-surgery correlated with clinical course of disease and that serum HER-2/neu levels were observed to increase with disease progression or to decrease with response to therapy. Several reports also show that elevated levels of serum HER-2/neu can occur in women with metastatic breast cancer that had primary breast tumors that were negative for HER-2/neu expression by immunohistochemistry. According to many IHC and serum studies, the HER-2/neu protein is overexpressed in many tumors of epithelial origin including lung, prostate, pancreatic, colon, stomach, ovarian, and hepatocellular cancer.

### *L-Asparaginase Treatment of Cancer Guided by a Biomarker*

L-asparaginase (L-ASP), a bacterial enzyme used to treat acute lymphoblastic leukemia, selectively starves cells that cannot synthesize sufficient asparagine for their own needs Studies show that cancer cells that contain less asparagine synthetase (ASNS) are more susceptible to L-ASP. The response to L-ASP therapy is often better when the expression of ASNS is limited. A new method has been described

for enhancing L-ASP activity by combining it with antagonists of ASNS, such as siRNAs, antisense nucleotides, antibodies or small-molecule inhibitors for treatment of cancer (Lorenzi et al. 2006). Reducing or suppressing the expression of ASNS potentiates the growth inhibitory activity of L-ASP four- to five-fold. Tissue microarrays confirmed low ASNS expression in a subset of clinical ovarian cancers as well as other tumor types. Overall, this pharmacogenomic/pharmacoproteomic study suggests the use of L-ASP for personalized treatment of a subset of ovarian cancers (and perhaps other tumor types), with ASNS as a biomarker for selection of patients most likely to respond to L-ASP treatment. The technology is currently in the preclinical stage of development. With respect to L-ASP treatment of patients with solid tumors, phase I clinical trials have been initiated using L-ASP in combination with gemcitabine.

## *Oncogene GOLPH3 as a Cancer Biomarker*

Genome-wide copy number analyses of human cancers have identified frequent 5p13 amplification in several solid cancers, including lung, ovarian, breast, prostate and melanoma. Using integrative analysis of a genomic profile of the region, a Golgi protein, GOLPH3, was identified as a candidate targeted for amplification (Scott et al. 2009). Gain- and loss-of-function studies in vitro and in vivo validated GOLPH3 as a potent oncogene. Physically, GOLPH3 localizes to the trans-Golgi network and interacts with components of the retromer complex, which has been linked to target of rapamycin (TOR) signaling. GOLPH3 regulates cell size, enhances growth-factor-induced mTOR (also known as FRAP1) signaling in human cancer cells, and alters the response to an mTOR inhibitor in vivo. Thus, genomic and genetic, biological, functional and biochemical data in yeast and humans establishes GOLPH3 as a new oncogene that is commonly targeted for amplification in human cancer, and is capable of modulating the response to rapamycin, a cancer drug in clinical use. A protein made from GOLPH3 may serve as a biomarker for tumors that can be effectively treated with the rapamycin: tumors with a high level of the protein are more apt to shrink in response to the drug than those with low levels.

## *Predictive Biomarkers for Cancer*

Unpredictable efficacy and toxicity are hallmarks of most anticancer therapies. Predictive markers are factors that are associated with response or resistance to a particular therapy. Currently, the only recommended predictive markers in oncology are estrogen receptor (ER) and progesterone receptor (PR) for selecting endocrine-sensitive breast cancers and HER-2 for identifying breast cancer patients with metastatic disease who may benefit from trastuzumab. For malignancies other than breast cancers, validated predictive markers are not available as yet.

### *Sequencing to Discover Biomarkers to Personalize Cancer Treatment*

A sequencing-based method for personalized analysis of rearranged ends (PARE) in individual tumors identifies biomarkers that could subsequently be used to track cancer using patient blood samples. The concept of the utilization of rearranged ends for development of personalized biomarkers has attracted much attention owing to its clinical applicability. Although targeted next-generation sequencing (NGS) for recurrent rearrangements has been successful in hematologic malignancies, its application to solid tumors is problematic due to the paucity of recurrent translocations. However, copy-number breakpoints (CNBs), which are abundant in solid tumors, can be utilized for identification of rearranged ends. The approach relies on massively parallel sequencing by the use of SOLiD platform (Life Technologies) to find translocations and rearrangements in solid tumors. After finding such rearrangements in breast and colorectal cancer (CRC) samples, further studies have used PARE to track cancer treatment response, recurrence, and metastasis in CRC patients. Targeted NGS at copy-number breakpoints (TNGS-CNB) has been used in CRC (Kim et al. 2014a). For deduction of CNBs, a novel competitive SNP (cSNP) microarray method was developed entailing CNB-region refinement by competitor DNA. Results indicate that TNGS-CNB, with its utility for identification of rearrangements in solid tumors, can be successfully applied in the clinical laboratory for cancer-relapse and therapy-response monitoring.

This is an important step in bringing NGS technologies to personalized patient care. Most tumors do not contain rearrangements, but the location of these rearrangements varies from one individual to the other, making them good biomarker candidates. Previous studies on sequencing individuals' genomes were focused on single-letter changes, but later studies looked for the swapping of entire sections of the tumor genome. Rearrangements were found in CRC genomes. Because most clinically important tumors contain DNA rearrangements, the PARE approach holds promise for finding patient-specific biomarkers that can be used to improve the treatment of a variety of cancers.

The cost of PARE was reportedly around $5,000 per assay, though the cost is expected to go down as sequencing prices drop and read quality and length improve. As PARE becomes affordable, it will be a helpful addition for physicians to tailor patient care and may become a useful supplement to traditional monitoring by imaging or other approaches. The method holds potential for monitoring cancer and guiding treatment, e.g. it may help differentiate between individuals whose cancers are cured by surgery alone and those who require follow-up with aggressive chemotherapy or radiation following surgery. PARE will be available for many cancer patients within 2–3 years, depending largely on sequencing costs.

### *VeraTag™ Assay System for Cancer Biomarkers*

The VeraTag™ assay system (Monogram Biosciences) is a high performance, high throughput system for studies of gene expression, protein expression and for applications such as cell signaling and pathway activation, protein-protein interaction and cell receptor binding. The system uses proprietary VeraTag™ reporters to multiplex the analysis of genes and/or proteins from the same sample. The VeraTag™ assay system is ideally suited to analysis of complex biology such as that seen in cancer. These unique assays can precisely measure many types of pathway biomarkers simultaneously – using small samples, such as those obtained from standard tumor biopsies. These biomarkers could be used to correlate disease type and progression, resulting in improved treatment. Novel VeraTag™ assays for unique protein biomarkers such as receptor-complexes and phosphorylation events are being developed to focus on profiling Epidermal Growth Factor Receptor (EGFR) family signal transduction pathways. Further research will be aimed at applying VeraTag™ assays to retrospective analysis of patient samples from clinical trials for validation and diagnostic development. It can accelerate the development of targeted therapeutics, improve clinical trial design and results, clarify and individualize the selection of medications, and optimize outcomes for patients with cancer. It can be used for developing companion diagnostics to guide selection of patients for HER-targeted therapies.

## Determination of Response to Therapy

Several approaches have been investigated for predicting and monitoring response to anticancer chemotherapy. Some of these are described here.

### *Biomarker-Based Assays for Predicting Response to Anticancer Therapeutics*

The high incidence of resistance to DNA-damaging chemotherapeutic drugs and severe side effects of chemotherapy have led to a search for biomarkers able to predict which patients are most likely to respond to therapy.

ERCC1-XPF nuclease is required for nucleotide excision repair of DNA damage by cisplatin and related drugs, which are widely used in the treatment of cancer. The levels of ERCC1-XPF in a tumor could indicate whether it will be sensitive or resistant to a certain chemotherapeutic agent. Although several commercially available antibodies are suitable for immunodetection of ERCC1-XPF in some applications, only a select subset is appropriate for detection of this repair complex in fixed specimens. A study provides reliable tools for clinicians to measure the enzyme

ERCC1-XPF as a biomarker in clinical specimens that could help stratify patients according to cancer risk, response to treatment and overall prognosis (Bhagwat et al. 2009).

Capecitabine (Roche's Xeloda) is a novel, oral fluoropyrimidine carbamate rationally designed to generate 5-fluorouracil preferentially in tumor tissue via a three-step enzymatic cascade. It belongs to the category of antimetabolites that stop cancer cells from making and repairing DNA, which is required for their growth. Companion diagnostic tests based on various biomarkers – thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase – are being investigated to predict responders to this therapy. Polymorphisms in DNA repair genes – AREG (amphiregulin) and the ERCC1 (excision repair cross-complementing group 1) – have been shown as promising predictive biomarkers of response to capecitabine-based chemoradiotherapy in locally advanced rectal cancer (Sebio et al. 2015).

## *Ex Vivo Testing of Tumor Biopsy for Chemotherapy Sensitivity*

Many oncologists are beginning to believe that new techniques to evaluate tumors' responses to chemotherapeutic agents promise a future of personalized cancer management. Rational Therapeutics' EVA® assay measures apoptotic events that occur as a result of drug exposure. Hence, highly responsive cancers are those with the greatest degree of apoptosis in the laboratory. The Company has developed a novel regimen for refractory ovarian cancers – gemcitabine plus cisplatin. Study results showed a correlation between ex vivo sensitivity and resistance and patient outcome. The Gynecologic Oncology Group, a multicenter non-profit organization sponsored by the National Cancer Institute, is conducting a national clinical trial of the gemcitabine plus cisplatin combination for treatment of relapsed ovarian cancer. The idea of the assays in predicting chemosensitivity continues to grow. It is not been used for first-line treatment for ovarian cancer yet because it has not been proved that anything is more effective than platinum and Taxol. But assays can provide valuable information for its selection as a second-line treatment. Lack of efficacy of the drug could be due to the drugs' inability to be delivered to the tumor or inappropriate levels of drug. In 50–60 % of the instances, a drug is not effective in vivo even though the in vitro assays predict efficacy.

ChemoFx Assay (Helomics) is an ex vivo assay designed to predict the sensitivity and resistance of a given patient's solid tumor to a variety of chemotherapy agents (Brower et al. 2008). A portion of a patient's solid tumor, as small as a core biopsy, is mechanically disaggregated and established in primary culture where malignant epithelial cells migrate out of tumor explants to form a monolayer. Cultures are verified as epithelial and exposed to increasing doses of selected chemotherapeutic agents. The number of live cells remaining post-treatment is enumerated microscopically using automated cell-counting software. The resultant cell counts in treated wells are compared with those in untreated control wells to

generate a dose-response curve for each chemotherapeutic agent tested on a given patient specimen. Features of each dose-response curve are used to score a tumor's response to each ex vivo treatment as "responsive," "intermediate response," or "non-responsive." Collectively, these scores are used to assist an oncologist in making treatment decisions.

## *Genomic Approaches to Predict Response to Anticancer Agents*

### Gene Expression Patterns to Predict Response of Cancer to Therapy

Human lymphoblastoid cells, immortalized white blood cell lines derived from different healthy individuals, display considerable variation in their transcription profiles, which underlies interindividual susceptibility to DNA damaging agents. Gene expression, measured by Affymetrix GeneChip Human Genome U133 Plus 2.0, has been associated with sensitivity and resistance to DNA-damaging anticancer agents (Fry et al. 2008). A cell line from one person would be killed dramatically, while that from another person can be resistant to exposure to the anticancer agent. Using computational models to pinpoint differentially expressed genes with positive or negative correlations, the investigators identified 48 genes whose pre-treatment expression could predict sensitivity to anticancer agent MNNG with 94 % accuracy. MNNG alkylates certain DNA bases, leading to mutagenesis. Some of this damage can be repaired by the DNA methyltransferase MGMT. But if it is not, the DNA mismatch repair or MMR pathway targets damaged DNA bases and sets off apoptosis. Consequently, cells with reduced MGMT activity but a functional MMR pathway are expected to be more sensitive to MNNG, whereas cells deficient in both pathways are more MNNG resistant but accumulate mutations when exposed to the compound. Because gene expression is the most accurate predictor of alkylation sensitivity, there are good prospects for translating these findings to a clinical setting to predict whether a tumor will respond to alkylation chemotherapy.

### Genomic Analysis of Tumor Biopsies

Genomic Health Inc is developing a service to provide individualized genomic analysis of tumor biopsies to physicians as a guide to treatment of patients with cancer. Fixed paraffin-embedded tissues (FPET), stored tumor tissue samples collected over the past 20 years, are used for this purpose. Instead of waiting years to accumulate fresh tissue and track patient outcomes, Genomic Health's FPET analysis can be performed using routinely stored biopsies from patients with known outcomes therefore accelerating clinical trials. RNA analysis of thin sections of standard tumor biopsies is used to evaluate panels of genes that may predict breast cancer recurrence and response to chemotherapy as well as response to EGFR inhibitor therapy in lung cancer. This approach is now being tested in clinical trials

on patients with breast cancer and lung cancer. This technology will allow physicians to tailor the treatment and prognosis for an individual patient, using a small panel of genes selected from thousands of genes.

Activating mutations of KIT or kinase platelet-derived growth factor receptor alpha (PDGFRA) are found in the vast majority of gastrointestinal stromal tumors (GISTs), and the mutational status of these oncoproteins is predictive of clinical response to imatinib. PDGFRA mutations can explain response and sensitivity to imatinib in some GISTs lacking KIT mutations.

### Genotype-Dependent Efficacy of Pathway Inhibition in Cancer

Therapeutic inhibition of genetically activated oncoproteins can induce massive apoptosis of tumor cells, which may lead to dramatic regression of cancer. The PI3K and MAPK signaling pathways are central regulators of oncogenic transformation and tumor maintenance. Systematic linking of drug response to genomic aberrations in NSCLC, as well as in cell lines of other tumor types and in a series of in vivo cancer models, has shown that tumors with genetically activated receptor tyrosine kinases depend on PI3K signaling, whereas tumors with mutations in the RAS/RAF axis depend on MAPK signaling (Sos et al. 2009). However, efficacy of downstream pathway inhibition is limited by release of negative feedback loops on the reciprocal pathway. By contrast, combined blockade of both pathways can overcome the reciprocal pathway activation induced by inhibitor-mediated release of negative feedback loops and results in a significant increase in tumor apoptosis. Thus, by using a systematic chemogenomics approach, genetic lesions connected to PI3K and MAPK pathway activation can be identified and provide a rationale for combined inhibition of both pathways. These findings may have implications for patient stratification in clinical trials.

### Mutation Detection at Molecular Level

It is known that genetic mutations are responsible for sensitizing some tumor cells to chemotherapy, while other mutations render tumor cells completely resistant to drug treatments. Research progress in this area has been slow because analysis of DNA from tumors is complicated by varying amounts of tumor cells in patient samples. Furthermore, the heterogeneous nature of many tumors makes it difficult to accurately sequence the tumor DNA, which is required in order to personalize treatment. This is compounded by cost-prohibitive, conventional low-resolution sequencing methods that lack sufficient accuracy to characterize the DNA in cancerous cells. Next generation sequencing can be used for the detection of cancer gene mutations present at extremely low levels. Microreactor-based 454 Sequencing™ (Roche) advanced sequencing technology can generate hundreds of thousands of DNA sequences in one run, rapidly and comprehensively conducting high-throughput nucleotide sequencing, with specific application to sequencing of

whole genomes and ultra-deep sequencing of target genes. The method is not only very sensitive, but it is also quantitative and provides a digital display of gene variation within tumors. It identifies rare cancer-associated genetic variations at the molecular level, potentially enabling the personalization of targeted therapies. This technology was used to analyze mutations in five exons of the EGFR gene in tumor samples from patients with lung cancer. The EGFR gene is the target for several new anticancer drugs called EGFR inhibitors. Thus 454 Sequencing may help to validate the ability of EGFR mutations to predict patient responsiveness to treatment with an EGFR inhibitor. Ultimately, this system will enable personalized medicine, such as identifying the early stages of drug resistance and facilitating a change in treatment that is tailored to a patient's unique genetic response.

#### RNA Disruption Assay™

RNA Diagnostics Inc has developed RNA Disruption Assay™ (RDA™) that enables determination of efficacy of chemotherapy within the first three cycles and helps in making decision about further continuation of therapy. The test is based on the observation that in some patients chemotherapy administration results in marked degradation of tumor RNA, indicating a positive response and tumor destruction.

#### Role of Genetic Variations in Susceptibility to Anticancer Drugs

Genetic variations in susceptibility to anticancer drugs has been investigated using a genome-wide model of human lymphoblastoid cell lines from the International HapMap consortium, of which extensive genotypic information is available (Huang et al. 2007). This model integrated genotype, gene expression, and sensitivity of HapMap cell lines to drugs. Associations were evaluated between genotype and cytotoxicity, genotype and gene expression and gene expression of the identified candidates was correlated with cytotoxicity. The analysis identified 63 genetic variants that contribute to etoposide-induced toxicity through their effect on gene expression. These include genes that may play a role in cancer (AGPAT2, IL1B, and WNT5B) and genes not yet known to be associated with sensitivity to etoposide. This method can be used to elucidate genetic variants contributing to a wide range of cellular phenotypes induced by chemotherapeutic agents.

### *Non-genetic Factors for Variations in Response of Cancer Cells to Drugs*

It is well known that not all cells of a particular cell type react to cancer treatments uniformly and genetics alone cannot explain sensitivity or resistance to chemotherapy. In the case of apoptosis mediated by TRAIL (tumor necrosis factor

(TNF)-related apoptosis-inducing ligand) it is common for some cells in a clonal population to die while others survive – a striking divergence in cell fate. Among cells that die, the time between TRAIL exposure and caspase activation is highly variable. Imaging of sister cells expressing reporters of caspase activation and mitochondrial outer membrane permeabilization after exposure to TRAIL has shown that naturally occurring differences in the levels or states of proteins regulating receptor-mediated apoptosis are the primary causes of cell-to-cell variability in the timing and probability of death in human cell lines (Spencer et al. 2009). Protein state is transmitted from mother to daughter, giving rise to transient heritability in fate, but protein synthesis promotes rapid divergence so that sister cells soon become no more similar to each other than pairs of cells chosen at random. These results have implications for understanding 'fractional killing' of tumor cells after exposure to chemotherapy and indicate that the genetic identity of a tumor cell is an incomplete predictor for how it will respond to certain treatments. These findings also offer an alternative to the cancer stem cell hypothesis, which states that certain cancers survive standard treatments because a population of tumor-specific stem cells evades chemotherapy or radiation. This study, however, offers an alternative explanation, i.e. that certain cells produce quantities of proteins purely through chance, which fundamentally alter the cell's response to treatment. This new insight will make it possible to design anticancer treatments that are more effective than those currently available.

### *Proteomic Analysis of Tumor Biopsies to Predict Response to Treatment*

Protein analysis of malignant tissue and the discovery of protein signatures have been used for assessing the stage of disease as well as their correlation with patient survival. Protein profiles have been obtained from human gliomas of various grades through direct analysis of tissue samples using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). Statistical algorithms applied to the MS profiles from tissue sections can identify protein patterns that correlate with tumor histology and patient survival. Protein patterns serve as an independent indicator of patient survival. This molecular approach to monitoring gliomas can provide clinically relevant information on tumor malignancy and is suitable for high-throughput clinical screening.

### *Real-Time Apoptosis Monitoring*

There is need for real-time monitoring of apoptosis because of the serious problems that result from not knowing if and when anticancer therapy starts to work. For the patient, receiving a therapy that is not effective means unnecessary suffering, both from the tumor continuing to grow and any side effects that accompany the

ineffective treatment. Receiving ineffective therapy for longer than needed also delays the start of second-line therapies that might work. Worse still, the failed treatment can trigger genetic defense mechanisms in tumor cells that can make it resistant to second-line therapies using other drugs. This phenomenon is known as cross-resistance.

The current months-long lag between the start of therapy and the appearance of obvious signs of initial success or failure also affects how new therapies undergo clinical testing. Because of the possibility of cross-resistance, FDA is reluctant to allow testing of new cancer therapies on anyone but those patients who have exhausted all other therapeutic possibilities. Unfortunately, such patients are far less likely to respond to any therapy, making it far more difficult to prove the benefits of an experimental therapy. This difficulty is particularly true for the new generation of molecularly targeted therapies that aim to stop tumor growth early in its progression. An available real-time apoptosis monitor might enable such drugs to be tested at the initial diagnosis of cancer with less concern that prolonged therapy, should it fail to work, would put patients at risk by letting their cancers grow unchecked for longer than necessary. Instead, getting an early sign that such an early therapy is not working would allow patients to receive conventional therapy more quickly. Recognizing such a need, the NCI's Unconventional Innovations Program is funding the development of an apoptosis detector.

## *Serum Nucleosomes as Indicators of Sensitivity to Chemotherapy*

In the nucleus of eukaryotic cells, DNA is associated with several protein components and forms complexes known as nucleosomes. During cell death, particularly during apoptosis, endonucleases are activated that cleave the chromatin into multiple oligo- and mononucleosomes. Subsequently, these nucleosomes are packed into apoptotic bodies and are engulfed by macrophages or neighboring cells. In cases of high rates of cellular turnover and cell death, they also are released into the circulation and can be detected in serum or plasma by Cell Death Detection-ELISAplus (Roche Diagnostics). As enhanced cell death occurs under various pathologic conditions, elevated amounts of circulating nucleosomes are not specific for any benign or malignant disorder. However, the course of change in the nucleosomal levels in circulation of patients with malignant tumors during chemotherapy or radiotherapy is associated with the clinical outcome and can be useful for the therapeutic monitoring and the prediction of the therapeutic efficacy.

In patients with inoperable small cell lung cancer, the efficacy of chemotherapy can be predicted early in the course of therapy by baseline values of serum nucleosomes as independent parameters. However, prediction of efficacy of chemotherapy in non-small cell lung cancer (NSCLC) requires consideration of the following:

- Staging
- Age

- Baseline value of biomarker CYFRA 21-1. In advanced stage NSCLC, the initial level of serum CYFRA appears to provide more prognostic information than clinical stage.
- Area under the curve (AUC) of the values of nucleosomes days on 1–8.

## *Targeted Microbubbles to Tumors for Monitoring Anticancer Therapy*

New strategies to detect tumor angiogenesis and monitor response of tumor vasculature to therapy are needed. Vascular Targeting Agent technology using contrast ultrasound imaging with microbubbles targeted to tumor endothelium offers a noninvasive method for monitoring and quantifying vascular effects of antitumor therapy. The microbubbles are tiny lipid or albumin shells filled with an inert gas that have a well-established safety record as contrast agents for ultrasound imaging applications, and they are currently widely used in cardiovascular medicine. Targeted microbubbles conjugated to MAbs were used to image and quantify vascular effects of two different antitumor therapies in pancreatic tumor-bearing mice treated with anti-vascular VEGF MAbs and/or gemcitabine (Korpanty et al. 2007). Video intensity from targeted microbubbles correlated with the level of expression of the target (CD105, VEGFR2, or the VEGF-VEGFR complex) and with microvessel density in tumors under antiangiogenic or cytotoxic therapy. It was concluded that targeted microbubbles represent a novel and attractive tool for noninvasive, vascular-targeted molecular imaging of tumor angiogenesis and for monitoring vascular effects specific to antitumor therapy in vivo. This information could allow oncologists to modify patient treatment regimens soon after starting therapy, so that nonresponders could be switched to other therapies that might be more effective for them. The clinical development of contrast agents is typically faster than for therapeutics, and clinical trials of this approach could be feasible within 12–18 months. The potential of the approach is enhanced by the fact that the targeted microbubbles are "read" using ultrasound technology, which is widely available in most physicians' offices and is minimally invasive, safe and cost-effective. The personalized medicine made feasible by this approach has the potential to increase the efficacy of cancer regimens, reduce side effects from ineffective treatments and improve the overall cost effectiveness of cancer therapy.

## *PET Imaging for Determining Response to Chemotherapy*

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) and cytosine arabinoside (cytarabine, ara-C) represent a class of nucleoside analogs used in cancer chemotherapy. Administered as prodrugs, dFdC and ara-C are transported across cell membranes and are converted to cytotoxic derivatives through consecutive phosphorylation

steps catalyzed by endogenous nucleoside kinases. Deoxycytidine kinase (DCK) controls the rate-limiting step in the activation cascade of dFdC and ara-C. DCK activity varies significantly among individuals and across different tumor types and is a critical determinant of tumor responses to these prodrugs. Current assays to measure DCK expression and activity require biopsy samples and are prone to sampling errors. Noninvasive methods that can detect DCK activity in tumor lesions throughout the body could circumvent these limitations. An approach to detecting DCK activity in vivo has been demonstrated by using positron emission tomography (PET) and $^{18}$F-labeled 1-(2′-deoxy-2′-fluoroarabinofuranosyl) cytosine] $^{18}$FFAC, a DCK substrate with an affinity similar to that of dFdC as a PET probe (Laing et al. 2009). In vitro, accumulation of $^{18}$FFAC in murine and human leukemia cell lines is critically dependent on DCK activity and correlates with dFdC sensitivity. In mice, $^{18}$FFAC accumulates selectively in DCK-positive vs. DCK-negative tumors, and $^{18}$FFAC microPET scans can predict responses to dFdC. The results suggest that $^{18}$FFAC PET might be useful for guiding treatment decisions in certain cancers by enabling individualized chemotherapy.

### PET Imaging with Tyrosine Kinase Inhibitors

Several small molecule tyrosine kinase inhibitors (TKIs) have been developed and approved. Treatment efficacies with TKI therapeutics are still too low and improvements require a personalized medicine approach. PET with radiolabeled TKIs (TKI-PET) is a tracking, quantification and imaging method, which provides a unique understanding of the behavior of these drugs in vivo and of the interaction with their target(s). An overview of tracer synthesis and development indicated that each TKI requires a tailor made approach (Slobbe et al. 2012). Moreover, current preclinical work and the first proof-of-principle clinical studies on the application of TKI-PET illustrate the potential of this approach for improving therapy efficacy and personalized cancer treatment.

## *Tissue Systems Biology Approach to Personalized Management of Cancer*

Cellular Systems Biology (CSB™) applied to tissues has been named “Tissue Systems Biology” (TSB™) and involves the use of panels of fluorescence-based biomarkers that report the systems read-out of patient samples. Cellumen Inc (parent company of Cernostics) has successfully applied CSB™ to drug discovery, drug development and personalized medicine over 3 years. As of September 2008, Cernostics Pathology was creating a complete digital imaging pathology platform by integrating the best available components, while building advanced informatics tools to manage, mine and classify patient tissue samples. The first diagnostic/therapeutic test being developed by Cernostics is a breast cancer test as part of collaboration with the Mayo Clinic.

## Molecular Diagnostics Combined with Cancer Therapeutics

Basics of combination of diagnostics with therapeutics are discussed in Chap. 2. Cancer is a good example of such a combination, which would be useful for personalized management of cancer. Approximately 800 oncology drugs, many of which target specific mutations, are currently in development, resulting in a growing need for new companion diagnostics. Examples of technologies that can be used to combine diagnosis and therapeutics for cancer are listed below and will be discussed further under personalized management of various cancers.

- AmpliChip P53 as companion diagnostic for the anticancer drug Nutlin
- Flow cytometry testing for minimal residual disease in CLL treated with Campath
- Abl mutations testing in CML for Gleevec-resistance
- EGFR mutations testing in NSCLC for treatment with Tarceva/Iressa
- 5q FISH testing in myelodysplastic syndrome for lenalidomide (Revlimid) therapy

### *Aptamers for Combined Diagnosis and Therapeutics of Cancer*

Aptamers (derived from the Latin word 'aptus' = fitting) are short DNA or RNA oligomers, which can bind to a given ligand with high affinity and specificity due to their particular three-dimensional structure and thereby antagonize the biological function of the ligand. Aptamers are beginning to emerge as a class of molecules that rival antibodies in both therapeutic and diagnostic applications. Aptamers are different from antibodies, yet they mimic properties of antibodies in a variety of diagnostic formats.

High affinity aptamers are being developed as targeted therapeutics for the diagnosis, imaging, staging and treatment of cancer. This method offers, apart from an immediate application in the diagnosis, imaging and treatment of breast and other epithelial cancers, a generic application for the treatment of neoplastic disorders and a potential for future development. Combinatorial libraries have been used for the selection of aptamers that bind to well-characterized and established cancer biomarkers selectively and with high affinity. As part of their design, the aptamers are conjugated to ligands, molecules bearing binding sites for metal ions, to impart the therapeutic and diagnostic properties. In particular, stable chelation of technetium, rhenium and yttrium radioisotopes result in novel radiopharmaceutical agents for imaging and selective cell kill as part of cancer diagnosis, imaging and therapy. The use of paramagnetic gadolinium produces a novel, targeted MRI contrast agent that can achieve high local concentrations around the tumor site, thus offering high definition imaging at lower gadolinium concentrations. The use of europium or terbium confers fluorescent properties to the aptamer complex, for use in diagnostic assays. These molecules offer significant advantages over existing antibody and peptide based recognition procedures in that they possess higher binding affinities to the

target leading to longer retention times and the ability to deliver a higher payload of the metal ion precisely to the target with a lower overall dose of the agent. The size of these molecules leads to reduced immunogenicity and increased tumor penetration, further enhancing their efficacy while minimizing potential side effects.

## *Combining Diagnosis and Therapy of Metastatic Cancer*

Biomarkers of metastases of various cancers have been investigated. Examples are given along with specific cancers. Examples are as follows:

CUB domain-containing protein 1 (CDCP1) is a transmembrane protein that is highly expressed in stem cells and frequently overexpressed and tyrosine-phosphorylated in cancer. CDCP1 promotes cancer cell metastasis. A study has shown that hypoxia induces CDCP1 expression and tyrosine phosphorylation in hypoxia-inducible factor (HIF)-2α–, but not HIF-1α–, dependent fashion (Emerling et al. 2013). shRNA knockdown of CDCP1 impairs cancer cell migration under hypoxic conditions, whereas overexpression of HIF-2α promotes the growth of tumor xenografts in association with enhanced CDCP1 expression and tyrosine phosphorylation. IHC analysis of tissue microarray samples from tumors of patients with clear cell renal cell carcinoma shows that increased CDCP1 expression correlates with decreased overall survival. Together, these data support a critical role for CDCP1 as a unique HIF-2α target gene involved in the regulation of cancer metastasis, and suggest that CDCP1 is a biomarker and potential therapeutic target for metastatic cancers.

Mena protein potentiates and modulates cellular migration and is found in the developing embryo where it plays an important role in the developing nervous system among other functions. It facilitates and organizes formation, extension and navigation of growing nerve fibers through tissue to link with other neurons, forming the proper circuits needed for a functional nervous system. However, in metastatic cancer cells, high levels of the Mena protein accumulate and influence a number of intracellular signaling programs. Mena facilitates a process whereby tumor cells send out a well-organized protuberance that invades surrounding tissue and pulls the remainder of the cell behind it. Mena modulates the strength and direction of this invasive process and steers the migrating cancer cell in the direction of blood vessels through its ability to modulate the metastatic cell's response to chemical signals that attract it to blood vessels. Mena is present in cancer cells in several isoforms that are similar but slightly different in structure. Despite similarity in structure, protein isoforms differ considerably in their influence on cells. MetaStat Inc has identified the most dangerous isoform of Mena named MenaINV (Mena invasive). Mena11A, on the other hand, is the Mena isoform that seems to exert a much more positive influence on the cell's behavior, reducing the ability of cells to break away from the tumor and invade and migrate toward blood vessels. Metastat's key discovery is that it can predict the metastatic potential of a cancer cell by measuring the relative levels of MenaINV and Mena11A. As the relative levels of

MenaINV rise and Mena11A fall the cancer cell transitions to a more metastatic shape and behavior. These metastasis promoting behavior changes include increased migratory behavior, changes in shape, loss of adhesion to neighboring cells, and up to 100-fold greater sensitivity to the chemical attractant that lures metastatic cells to blood vessels.

In 2013, MetaStat Inc signed two licensing agreements – one with the Massachusetts Institute of Technology and the other with Montefiore Medical Center, Bronx, New York – for the use of alternatively spliced mRNA and Mena protein isoform biomarkers for diagnosis, prognosis, as well as treatment of metastases of solid epithelial cancers. This platform directly links a therapeutic to its companion diagnostic based on the detection and targeting of alternatively spliced oncogenes, which drive tumor progression and resistance, thereby offering a unique opportunity for personalized treatment of cancer.

## *Detection and Destruction of CTCs with Nanoparticles and X-Rays*

Early detection and eradication of circulating tumor cells (CTCs) is important for the management of cancer metastases. A technique for detection and killing of CTCs has been described that uses magnetic and bismuth nanoparticles, X-ray fluorescence spectrometry, and X-rays (Hossain et al. 2012). Nanoparticles are modified with tumor targeting agents and conjugated with tumor cells through folate receptors over-expressed on cancer cells. A micro-magnet is used to collect CTCs suspended inside a flowing medium that contains phosphate buffered saline or whole blood. Characteristic X-ray emissions from collected bismuth nanoparticles, upon excitation with collimated X-rays, are used to detect CTCs. Dose of primary X-rays can be enhanced to kill the localized CTCs in vivo by radiation-induced DNA damage, enabling personalized cancer management.

## *Monoclonal Antibodies for Combining Diagnosis with Therapy of Cancer*

Monoclonal antibodies (MAbs) can be used both for diagnosing and targeting cancer and some examples were given in Chap. 2. Two tests – Poteligeo Test IHC (immunohistochemistry) and Poteligeo Test FCM (Kyowa Medex) – were approved in Japan in 2012 as companion diagnostics for mogamulizumab (Kyowa Hakko Kirin's Poteligeo) injection, a therapeutic MAb for treatment of adult T cell leukemia (ATL). Poteligeo binds to CCR4, which is expressed on the surface of ATL cells, which are killed by MAb-dependent cell-mediated cytotoxicity. The companion diagnostic tests detect the presence of CCR4 expressed by ATL cells before

treatment with Poteligeo to enable identification of patients who would benefit from the drug. Poteligeo Test IHC is for use on tissue samples, such as lymph nodes whereas Poteligeo Test FCM uses flow cytometry to analyze blood samples from patients.

## Molecular Profiling of Cancer

Profiling of the 60 human cancer cell lines (the NCI-60) is being used by the NCI's Developmental Therapeutics Program (DTP) to screen >100,000 chemically defined compounds and natural product extracts since 1990. In statistical and machine-learning analyses, the screening data have proved rich in information about drug mechanisms of action and resistance. The NCI-60 panel already constitutes by far the most comprehensively profiled set of cells in existence, and much more molecular profile information on them is coming. The data have already yielded considerable biological and biomedical insight, but we have only scratched the surface thus far. The real value is realized when biomedical scientists with particular domain expertise are able to integrate and use the information fluently for hypothesis generation, hypothesis-testing. Given the large drug activity database, the NCI-60 cell line panel provides a unique opportunity for the enrichment of pharmacologic hypotheses and for advances toward the goal of personalized medicine (Weinstein 2006).

## Targeted Cancer Therapies

Targeted cancer therapy means selective action against molecular targets expressed in tumors. Conventional small-molecular therapy is usually targeted through selective action on the molecular machinery of the targeted cells. Targeted therapy also refers to screening patients so as to increase effectiveness of some form of therapy. Targeting reduces failure in both the drug development clinical research as well as postmarketing phases.

### *Targeting Glycoproteins on Cell Surface*

The biochemical signature that distinguishes cancer cells from normal cells is often carried on the outside of the cell membrane in the form of glycoproteins. These cell surface proteins are decorated with sugar chains in distinctive arrangements (or epitopes) that serve as therapeutic targets (or antigens) for agents such as monoclonal antibodies. Carbohydrates are also promising candidates for cancer control because they are present on cell surface and act as identification tags, through which they

can interact with their surroundings. Interfering with the normal cell recognition phenomenon using a small or large sugar molecule has been shown to block the progression of tumors by blocking angiogenesis, cell-to-cell matrix interactions and tumor invasion.

### *Targeting Pathways in Cancer*

The phosphatidilinositol 3-kinase/protein kinase B (PI3K-AKT) pathway presents an exciting new target for molecular therapeutics. PI3K-AKT pathway regulates a broad spectrum of cellular processes, some of which are necessary to maintain normal physiological functions and explain the toxicity of the drugs targeting the pathway. Elucidation of the precise function of the PI3K-AKT isoforms, could promote the development of isoform specific approaches to provide a selective action on tumor cells. Inhibition of the PI3K-AKT pathway at multiple sites or a combination with inhibitors of different signaling pathways may allow the development of an acceptable therapeutic index for cancer management. Further, inhibition of the PI3K-AKT pathway combined with conventional chemotherapy or radiation therapy may provide a more effective strategy to improve patient outcome. As molecular therapy targets the underlying defects in tumors, molecular diagnostics are required to identify patients with particular genetic aberrations in the pathway to enable personalized cancer treatment.

### *Targeted Personalized Anticancer Medicines in Clinical Use*

Several cancer therapies are targeted to specific mutations or receptors in tumors and have companion diagnostics to personalize their use. Table 10.3 shows marketed personalized anticancer drugs described later in this chapter under organs involved.

## Immunotherapy of Cancer

Cancer immunology deals with the study of natural interplay between oncogenesis, inflammation and immune surveillance of the body as well. Immune mechanisms may contribute to the efficacy of some currently used chemotherapeutic agents that may involve recognition of tumor-associated antigens or controlling growth of cancer stem cells. Immunological biomarkers may be used to determine prognosis of cancer and predict the efficacy of anticancer action. Important methods of immunotherapy for cancer are cytokines, monoclonal antibodies (MAbs), vaccines, and immunogene therapy.

**Table 10.3** Marketed anticancer personalized medicines

| Medicine/company | Target | Companion diagnostic (company) | Indication |
|---|---|---|---|
| Cetuximab (Erbitux)/ Merck KGaA | EGFR | TheraScreen K-RAS Mutation Kit (DxS/ QIAGEN) to exclude non-responders | Squamous cell carcinoma (head and neck), colorectal carcinoma |
| Crizotinib (Xalkori)/ Pfizer | ALK | ALK Break Apart FISH Probe (Vysis) to detect rearrangements of the ALK gene | Non-small cell lung cancer |
| Dasatinib (Sprycel®)/ Bristol-Myers-Squibb | BCL-ABL | Not required | Chronic myelogenous leukemia |
| Erlotinib (Tarceva)/Roche | EGFR | Cobas EGFR Mutation Test (Roche) | Non small cell lung cancer |
| Gefitinib (Iressa)/Astra Zeneca | EGFR | EGFR mutation test (DxS/ QIAGEN) to identify patients most likely to benefit | Lung adenocarcinoma |
| Imatinib (Glivec)/ Novartis | BCL-ABL, KIT | No companion test for leukemia, but c-KIT immunoassay for patients with gastrointestinal stromal tumors | Chronic myelogenous leukemia<br>Gastrointestinal stromal tumor |
| Nilotinib (Tarceva)/ Novartis | BCL-ABL | Not required | Chronic myelogenous leukemia |
| Panitumumab (Vectibix )/ Amgen | KRAS | Home-brew tests for the K-ras mutation | Colorectal cancer |
| Sorafenib (Nexavar)/ Bayer Healthcare | RAF, VEGF, KIT, FLT3 | Measuring levels of biomarkers, VEGF and KDR (VEGFR2), to determine response to sorafenib treatment (Bayer) | Renal cell carcinoma<br>Liver cancer |
| Trastuzumab (Herceptin)/ Roche | HER2 | PathVysion HER-2 breast cancer test kit (Vysis/ Abbott) | Breast carcinoma |
| Verumafenib (Zelboraf)/ Roche | BRAF | Multiplex PCR-based diagnostic for the BRAF V600E gene (Roche) | Melanoma |


*ALK* anaplastic lymphoma kinase

## *Functional Antibody-Based Therapies*

Functional antibodies are biological molecules that trigger death in cancer cells but not healthy cells. Functional antibodies target molecules carried on the outside of a cancer cell membrane known as antigens. These cell surface proteins are decorated with sugar chains in distinctive arrangements that can be used as targets for

therapeutic monoclonal antibodies. Antigens can act as biochemical signatures or markers that distinguish a cancer cell from a normal cell, and one person's cancer from another's. The selection of antibodies of interest at ARIUS Research Inc is based on the ability of the antibody to activate an antigen to selectively produce cancer cell death. ARIUS holds proprietary and patented technology that allows it to generate large numbers of functional antibodies at low cost. The core of ARIUS' technology platform is its high-throughput functional screens, which enable the Company to rapidly identify and select MAbs that show superior cancer killing ability. These MAbs, earmarked as potential drug candidates, are reserved for ARIUS' functional antibody library. The ARIUS approach to antibody development offers the following advantages:

- The process can produce multiple antibody drug candidates for any solid tumor cancer type
- The antibodies are functional in and of themselves and are drug candidates at the very outset
- Development is more flexible and less expensive because the antibodies are derived from patient tumors and do not rely on isolated target antigens that may have to be in-licensed
- The antibodies can be used to discover novel cancer antigens, since they are not produced against pre-defined targets

The developmental tasks remaining are similar to classic antibody development pathways with the exception of finding the target for the newly formed functional anticancer antibody. Generally, a number of biochemical and proteomic approaches are taken for the identification of the target antigen. In addition, a number of validation studies for the antibody are performed including testing for recognition of human cancer, as well as specificity studies. The antibodies are studied in animal models of human cancer to determine its effectiveness in vivo. If the antibodies are found to be safe and effective then they become candidate for clinical study. ARIUS has built a library of over 100 functional antibodies, plus attendant protocols and a database. Three of these are in animal testing phase:

1. ARvitamab (ARH460-23) suppresses tumor growth with the following feature: (a) prevents metastases in human lung cancer models; (b) is compatible with and additive to cisplatin chemotherapy to improve disease free survival; (c) recognizes a widely distributed tumor-associated antigen; and (d) is non-toxic in animal models. The putative target antigen for ARvitamab exhibits increased expression in many cancers including those involving breast, pancreatic, colon and prostate as well as nonepithelial cancers such as melanoma and lymphoma.
2. ARH460-16-2 displays cytotoxic activity in vitro against human breast cancer, colon cancer and melanoma cell lines. More significantly, it showed almost complete suppression of tumor growth in a prophylactic breast cancer xenograft model while the antibody was being administered. Ongoing research and development is aimed at identifying and validating the ARH460-16-2 target, establishing the safety and specificity of the antibody and producing a humanized form of the antibody for human clinical trials.

3. ARH460-22-1 has cytotoxic activity in vitro against human breast cancer, colon cancer and melanoma cell lines. Ongoing research is aimed at identifying and validating the antibody target, and showing antibody safety and specificity. A humanized version of this antibody is currently under development.

The long-term aim of targeted antibody therapy is to match multiple antibodies to different antigens on each patient's cancer cell, delivering multiple cancer killing messages simultaneously. Personalized therapy will improve on targeted therapy by further reducing the risks of failed treatment and improving the likelihood of cure.

Genentech's anticancer drug Herceptin may be considered the first targeted antibody therapy in that it is only appropriate for use in patients who over-express the Her2-Neu antigen on the surface of their breast cancer cells.

## *Immunotherapy of Dormant Cancer*

Clinical cancer dormancy is evident from the detection of circulating tumor cells (CTCs) in the blood and tissue-residing disseminated tumor cells in the bone marrow of cancer survivors who have been clinically disease free. Emerging evidence from clinical and preclinical studies suggests that tumor dormancy is a critical step in the development of both primary cancer and advanced-stage disease. A review has shown that (i) naturally occurring tumor dormancy precedes occurrence of primary cancer; and (ii) conventional cancer therapies result in treatment-induced tumor dormancy, which in turn could lead to distant recurrence of cancer or permanent tumor dormancy, depending on immunogenic status of dormancy (Manjili 2014). Given that cellular dormancy is an evolutionary conserved survival mechanism in biologic systems, any stress or cytotoxic therapy could trigger cellular dormancy. Therefore, a successful cancer therapy is likely to be achieved by establishing permanent tumor dormancy and preventing distant recurrence of cancer or by eliminating dormant tumor cells. This could be accomplished by cancer immunotherapy because of the establishment of long-term memory responses.

Mechanisms involved in metastatic cancer dormancy – cellular dormancy, angiogenic dormancy, and immune-mediated dormancy – can restrain disseminated cancer cells, thereby promoting their permanent dormancy (Romero et al. 2014). CD8+ T lymphocytes play a relevant role in maintaining immune equilibrium with metastatic dormant cells, and MHC class I surface expression on tumor cells may also be involved. NK cells have an activator function that triggers a CTL response. Furthermore, immune dormancy promotes cancer cell growth arrest and angiogenic control.

Immunotherapeutic interventions in metastatic dormancy may help to control or eradicate cancer. Activation or increase of the CTL immune response or reversal of cancer cell-induced CTL immunosuppression might be useful for restraining or destroying metastatic cells. These objectives may be achieved by recovering or increasing MHC class I surface expression on cancer cells or by activating NK cells. Thus immune-mediated metastasis dormancy provides an opportunity for targeting cancer by immunotherapy.

## Personalized Cancer Vaccines

There are several types of cancer vaccines, which include nucleic acid-based, MAb-based and cell-based vaccines. Various types of cells are used including tumor cells and dendritic cells. Combination of different methods and genetic modification of cells are also used. Personalized vaccines may be antigen-specific or tumor-derived, but patient-specific vaccines may be a combined approach. Most of the personalized cancer vaccines are cell-based and these were the earliest forms of personalized medicine (Jain 2010). A true personalized vaccine is one in which patient's own cells are used.

### *Antigen-Specific Vaccines*

Antigen-specific approach may generate an antigen-specific response even when the tumor antigens are not known. Currently the scope of cancer immunization is limited because most of the vaccines have targeted antigens that are restricted to a subset of patients. Functional genomics and proteomics will enable molecular characterization of whole transcriptomes and proteomes of cancer cells, thereby also identifying potential new targets for cancer immunotherapy. Based on fundamental immunological knowledge, the most promising approach would be patient-tailored.

#### Active Immunotherapy Based on Antigen Specific to the Tumor

Active immunotherapy is focused on overcoming the limitations of the immune system and directing it to mount an attack against cancer cells. Activating the immune system begins with the selection and modification of a tumor antigen specific to the cancer (e.g. prostatic acid phosphatase found in ~95 % of prostate cancers), which is produced using recombinant DNA technology.

The lead product in this category is sipuleucel-T (Provenge™, Dendreon Corporation), which targets prostatic acid phosphatase. A proprietary technique is then used to isolate antigen presenting cells taken from a cancer patient, which are combined with the modified antigen using Antigen Delivery Cassette™. The activated cells are then re-administered to the patient to stimulate T-cells to recognize and attack cancer cells that contain prostatic acid phosphatase. Sipuleucel-T has been approved by the FDA for the treatment of patients with early-stage and advanced prostate cancer. In clinical studies, patients typically received 3 infusions over a 1-month period as a complete course of therapy. Integrated results of two randomized trials demonstrate a survival benefit for prostate cancer patients treated with sipuleucel-T with a modest toxicity profile (Higano et al. 2009). A phase III clinical trial, IMPACT (IMmunotherapy for Prostate AdenoCarcinoma Treatment)

found that sipuleucel-T reduced the risk of death by 22.5 % compared with a placebo. The treatment extended the lives of patients by 4–5 months and 33 % percent of patients with advanced disease were still alive 3 years after treatment with sipuleucel-T. Although sipuleucel-T is prostate-specific, the underlying principle may be applicable to other cancers and it may be used in combination with chemotherapy. Several MAbs are in preclinical development, which are designed to recognize a specific antigen present on tumor cells but not on healthy cells and bind to that antigen to cause the death of the tumor cell. By this approach healthy cells should not be affected, reducing or eliminating the harsh side effects of many conventional cancer therapies.

GlaxoSmithKline is developing MAGE (melanoma antigen gene)-A3, a tumor antigen-based patient-specific vaccine for melanoma and it has undergone phase II clinical trials. Distinct gene expression profiles have been identified on pretreatment biopsies that are associated with a positive or negative clinical outcome, and this might be useful as a predictive biomarker for clinical trials of melanoma vaccines (Gajewski et al. 2010). Phase II clinical trials have demonstrated a clinical benefit by postoperative vaccine with MAGE A3 in non-small cell lung cancer and in stage IV melanoma, which have led to the current phase III trials (Peled et al. 2009).

### *Tumor-Derived Vaccines*

Although cancers may arise by common mechanisms, i.e. through mutations in genes implicated in cell transformation (i.e. p53, ras), they undergo additional random mutations in other genes. These mutations lead to expression of foreign antigens, forming a molecular "fingerprint" that uniquely characterizes the patient's tumor. Because mutations are generated randomly, the antigenic fingerprint of one person's cancer can never be duplicated in another person's cancer. Thus individual cancers within the same pathological category are antigenically distinct. This fundamental property requires that each patient's immune system be trained to recognize that patient's specific cancer. This is the basis of manufacture of cancer immunotherapeutic from each patient's own tumor tissue. Another approach is to identify as many candidates as possible for tumor-associated T-cell epitopes in individual patients. Expression profiling of tumor and normal tissue can be performed to identify genes exclusively expressed or overexpressed in the tumor sample.

Mass spectrometry enables characterization of several different major histocompatiblity complex (MHC) ligands from the same tumor sample. Combining these two analytic tools, it is possible to propose several candidates for peptide-based immunotherapy. This integrated functional genomics approach can be used for the design of antitumor vaccines tailored to suit the needs of each patient.

Whole tumor cells of the patient, rendered safe by irradiation and mixed with an immunological adjuvant, were one of the earliest forms of personalized cell therapy. This approach avoids the need for tumor antigens to be identified before treatment and allows all of the relevant antigens to be included in the vaccine. Initial clinical

studies showed the safety of this approach, with side effects mainly limited to local reactions at the site of the vaccine injection. Immunogenicity of tumor cell vaccines can be improved by transducing the tumor cell with genes that encode key components of the immune response, e.g. cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and costimulatory molecules.

Most of the cancer vaccines are being developed in the commercial sector. Whole tumor vaccines have gone through clinical trials. None of the tumor cell vaccines are in the market in the US.

### FANG Vaccine

The FANG™ vaccine (Gradalis Inc) is an autologous tumor-based product incorporating a plasmid expressing a well-established immune activator, GM-CSF and a novel bifunctional short hairpin RNAi (bi-shRNAi) targeting furin convertase, thereby downregulating endogenous immunosuppressive TGF β1 and β2. It is manufactured from a cell suspension derived from a portion of a patient's tumor removed during surgery. bi-shRNAi is introduced into the cells by electroporation. Cells are then incubated overnight, irradiated, frozen, tested and released. Vaccine is shipped to the patient's clinic where doses are thawed and administered monthly by intradermal injection. FANG manufacturing is a straightforward 2-day cGMP process that is applicable to nearly all tumor types with no modification, and it does not require patients to undergo apheresis or other treatments except surgical tumor removal if indicated.

Results of a phase I study showed that treatment with FANG was safe and significantly increased survival in patients with advanced stage cancer compared to patients who received other forms of treatment (Senzer et al. 2012). The vaccine elicits a robust and lasting immune response, resulting in statistically-significant prolonged survival in patients with advanced stage disease. Currently, FANG is being evaluated in several phase II trials in patients with advanced ovarian cancer, advanced melanoma and advanced colorectal cancer with liver metastases. In addition, Gradalis has initiated a clinical program evaluating FANG in children with Ewing's sarcoma.

### MyVax

MyVax® (Genitope Corporation) is an investigational treatment based on the unique genetic makeup of a patient's tumor and is designed to activate a patient's immune system to identify and attack cancer cells. It combines a protein derived from the patient's own tumor with an immunologic carrier protein and is administered with an immunologic adjuvant. Development of this immunotherapeutic approach has been limited by manufacturing difficulties. Genitope has developed a proprietary manufacturing process that overcomes many of these historical manufacturing limitations. A phase II trial found that immunization of follicular lymphoma patients

with MyVax is safe and patients often mount tumor-specific immune responses (Timmerman et al. 2009). These results form the basis of a current pivotal phase III trial of MyVax in follicular non-Hodgkin's lymphoma.

### OncoVAX

OncoVAX® (Vaccinogen Inc) is a vaccine from the patient's own tumor with or without fresh frozen Bacillus Calmette-Guerin as an adjuvant. The cells are dissociated, irradiated to make them non-tumorigenic and administered to the patient by three weekly injections, starting 4 weeks after surgery. A booster vaccination is administered 6 months later. OncoVAX® is administered to patients with colon cancer after surgery to reduce recurrence and deaths. Results of a phase III clinical trial showed significantly improvement in 5-year overall survival and recurrence-free survival in stage II colon cancer and some benefits in stage III colon cancer (Hanna et al. 2006). This study was accepted by the FDA as supportive data for the next phase IIIb clinical trial where disease-free survival will be used as a clinical endpoint for the interim analysis.

### Tumor Cells Treated with Dinitrophenyl

M-Vax (AVAX Inc) is a vaccine based on modification of autologous tumor cells with the hapten, dinitrophenyl (DNP), which binds to molecules on the surface of cells and helps trigger immune responses. DNP-treated cancer cells are combined with an adjuvant, bacillus Calmette-Guerin, and the vaccine is injected intradermally into cancer patients. The patient's immune system is then better able to recognize, locate and combat remaining cancer cells that may have metastasized to other areas of the body. It is these remaining cancer cells that, if left undetected and untreated, can potentially form additional cancerous tumors and eventually lead to death. Immune responses help the body determine which foreign proteins to attack. The ability of DNP to modify proteins and render them more easily identified as foreign to the immune system has been well documented over the past thirty years. Clinical trials have been conducted in stage III melanoma patients with a 5-year survival rate was 44 % as compared to 20–25 % with surgery alone (Berd 2004). This vaccine may help prevent cancer recurrence and increase the long-term survival rate of patients with other cancers as well. O-Vax is in phase II clinical trials for ovarian cancer.

### Prophage

Prophage (vitespen, Agenus) is a patient-specific and tumor-specific therapeutic cancer vaccine, which contains the heat shock protein, gp96, and associated peptides that are purified from the patients' own tumor tissue (Wood and Mulders

2009). Following surgery to remove a part or whole of the tumor, the tissue specimen is shipped frozen to Agenus, which prepares the vaccine and sends it back for intradermal injection when the patient has recovered from surgery. It has been tested in numerous patients in multiple cancers in clinical trials and approved in Russia as Oncophage® for the adjuvant treatment of kidney cancer patients at intermediate-risk for disease recurrence. It has orphan drug designation from the FDA as well as EMEA for kidney cancer and glioblastoma. Results of clinical trials of Prophage show that:

- It is well tolerated
- Elicits tumor-specific T cell responses and innate immune response irrespective of tumor type
- Efficacy is most significant in patients with early-stage disease and low tumor burden

#### Melacine

Melacine melanoma vaccine was developed by Corixa Corporation (now acquired by GlaxoSmithKline) consists of lysed cells from two human melanoma cell lines combined with an adjuvant that includes monophosphoryl lipid A and mycobacterial cell wall skeleton, both of which activate the human immune system. Melacine vaccine is approved in Canada but not in the USA. It is administered as a two-shot vaccination delivered as four 6-month cycles, each consisting of 10 treatments followed by a 3-week rest. Patients who respond are maintained on long-term therapy.

A randomized phase III trial of Melacine plus low-dose IFN-α2b in malignant melanoma had an effect comparable to standard high-dose IFN-α2b but with less toxicity (Ding and Wei 2007). Analysis of clinical benefit following completion of the data sweep in patients who were positive for expression of either Class I MHC HLA A2 or C3 genes continued to show a highly statistically significant clinical benefit of Melacine in terms of increased disease free survival. Patients with these genes account for an approximate 60–70 % of all melanoma patients.

### *Patient-Specific Cell-Based Vaccines*

#### Dendritic Cell-Based Vaccines

Dendritic cells (DCs), named after their long arms, comprise a system of leukocytes widely distributed in all tissues. DCs are derived from bone marrow progenitors and circulate in the blood as immature precursors prior to migration into peripheral tissues. Within different tissues, DCs differentiate and become active in the taking up and processing of antigens and their subsequent presentation on the cell surface linked to major histocompatibility complex (MHC) molecules. Upon appropriate stimulation, DCs undergo further maturation and migrate to secondary lymphoid

tissues where they present antigens to T cells and induce an immune response. Dendritic cells can be derived from CD34+ precursors in response to granulocyte macrophage colony stimulating factor and tumor necrosis factor and from monocytes cultured with granulocyte macrophage colony stimulating factor and interleukin-4. DCs have the capacity to prime tumor-specific T cell responses and are considered to be potentially effective vaccines for immunotherapy of cancer.

Various approaches for ex vivo loading of DCs with tumor-specific antigens include tumor-derived peptide/protein, RNA/DNA, necrotic tumor cells, chaperone proteins, exosomes, and or tumor cell-DC fusion (Janikashvili et al. 2010). DCs may be administered intravenously, intradermally, subcutaneously or by intranodal or intratumoral injection.

**DCVax** (Northwest Biotherapeutics) is a personalized therapeutic cancer vaccine manufactured from the patient's own DCs that have been modified to teach the immune system to recognize and kill cancer cells bearing the biomarker of patient's tumor. Published clinical trials of DC vaccine for high-grade glioma patients suggest favorable clinical outcomes with evidence of low toxicity in effective induction of antitumor immunity correlating with clinical improvement (Wheeler and Black 2009). Preliminary reports on DCVax-Brain clinical outcomes seem to follow these trends. DCVax-Brain has been granted an Orphan Drug designation and received clearance from the FDA to commence a phase II clinical trial for glioblastoma multiforme. DCVax-Lung has received clearance from the FDA for phase I trials.

**Imetelstat** (Geron Corporation's GRNVAC1) is a therapeutic cancer vaccine comprised of autologous DCs loaded ex vivo with telomerase reverse transcriptase (hTERT) mRNA. hTERT represents an attractive target for cancer immunotherapy because it is overexpressed in most human tumors. Imetelstat is injected into intradermally from where the dendritic cells travel to the lymph nodes and instruct cytotoxic T-cells to kill tumor cells that express telomerase on their surface. Results of the first completed phase I/II clinical trial of Imetelstat in metastatic prostate cancer patients showed that the vaccine was well tolerated with no major treatment-related toxicities (Su et al. 2005). In addition, telomerase specific T-cell responses were generated in 19 of 20 subjects and vaccination was associated with a statistically significant increase in PSA doubling time and clearance of prostate cancer cells from the patients' blood, indicative of potential clinical response. Imetelstat is a potent and specific telomerase inhibitor and so far the only drug of its class in clinical trials (Röth et al. 2010).

**Vaccines Based ON Genetically Modified Dendritic Cells** DCs, that have been generated in vitro and transduced with genes coding for tumor-specific antigens or pulsed with tumor specific antigen or peptide, could be useful for induction of cytotoxic T cell responses. Genetic engineering of DCs to express immunosuppressive or immunoregulatory molecules may provide a novel method to promote graft tolerance, reducing dependence on systemic immunosuppression.

Gene therapy techniques can be applied to DC vaccines using recombinant nonreplicating viral vectors to provide efficient and reliable means of gene transfer. Genetic material is introduced into DCs to provide them a renewable source of

antigen for presentation; this should lead to more sustained expression of antigen. The expression of viral (and therefore foreign) genes may boost the immune response, but this antiviral immunity primed by DCs may cause the immune system to destroy DCs rapidly in subsequent rounds of immunization. One solution may be to use viral vectors that do not result in the expression of viral genes, such as retroviruses or "gutless" adenoviral vectors. Adeno-associated viruses can be used to transduce human DCs and their main advantage is a decrease in viral-derived epitopes leading to decreased immunogenicity of the vector.

Lentivirus vectors can be used for genetic modification of human DCs and they have an advantage over retroviral vectors that they do not require target replication for efficient transduction. Approaches facilitating generation of DC vaccines for clinical trials and enhancing their viability, biodistribution, and capacity to stimulate antigen-specific immune responses are critical for immunotherapy. In one study, mouse bone marrow cells were programmed with lentiviral vectors so that they produced GM-CSF and IL-4 in an autonomous manner (Koya et al. 2007). Mice vaccinated with genetically modified DCs self-differentiated in vitro or in vivo and produced potent antigen-specific responses against melanoma, which correlated with protective and long-term therapeutic anticancer effects. Thus, DC precursors can be genetically engineered after a single ex vivo manipulation, resulting in DC vaccines with improved activity.

**Fusion of Tumor Cells with Dendritic Cells to Produce Cancer Vaccines** In this approach, a product is created using a technique that fuses the patient's own tumor cells with powerful, immune-stimulating DCs. The fusion product is then injected back into the patient with the goal of sparking a specific immune response against the cancer. This individualized cell therapy presents the full complement of antigens specific to the patient's tumor.

The BIOVAXID™ (Accentia BioPharmaceuticals) cancer vaccine evokes the power of each patient's immune system and primes it to recognize and eliminate malignant lymphoma cells, while sparing normal B cells. In this individualized therapy, cells are harvested from a patient's lymph node, and the unique cancer biomarkers on the outside of their cancer cells are identified. To create this idiotype vaccine, the antigen-bearing tumor cells are fused to antibody-producing mouse cells that act as mini-factories, churning out large quantities of the protein antigens, which are then given back to patients with an immune system booster. By priming the immune system with this antigen in the form of an autologous vaccine, the vaccine induces an immune response against the cancerous cells and creates an immune memory. Because it is derived from individual patient's cancer cells, the vaccine is a true targeted, personalized therapy. The vaccine's anticancer effect is different than non-targeted traditional therapy, as it arises from the immune system's defense cells' innate ability to selectively target foreign antigens. Moreover, the immune response triggered by the vaccine against the cancerous tissue is a natural disease-fighting mechanism and is associated with minimal toxicity. Phase I and II clinical trials demonstrated the immunogenicity, safety and therapeutic efficacy of BiovaxID (Reinis 2008). It is in phase III clinical trials at M. D. Anderson Cancer Center (Houston, TX) for follicular lymphoma.

**Concluding Remarks About DC-Based Vaccines** DC-based cancer vaccines are a major focus in cancer immunotherapy as the primary functions of DCs are the initiation and regulation of immune responses. However, some tumors may stop responding to DC-based vaccines due to development of immune tolerance, which can be overcome by personalized DC-based cancer vaccines as they contain nearly all the antigens in a tumor. Combination with chemotherapy may also be helpful by elimination of cancer cells and inhibition of inhibit tumor-induced suppressive factors.

## *Adoptive Cell Therapy*

Adoptive cell therapy (ACT), also called adoptive immunotherapy, is the isolation of antigen-specific T lymphocytes, their ex vivo expansion and activation, and subsequent administration in large numbers to the autologous host. It is a promising approach to inducing antitumor immune responses. The molecular identification of tumor antigens and the ability to monitor the persistence and transport of transferred cells has provided new insights into the mechanisms of tumor immunotherapy. Several studies have shown the effectiveness of ACT for the treatment of patients with selected metastatic cancers. Important features of studies on this topic are:

- Preclinical models have identified characteristics of lymphocyte cultures that are required for successful ACT therapy.
- The most important characteristic is the presence of high affinity, tumor-antigen-specific CD8+ T cells. There is generally a direct correlation between treatment efficacy and the number of transferred, tumor-specific cells.
- Preclinical models have also identified ways to manipulate the host immune environment that increase ACT therapeutic efficacy. These include immunosuppression before cell administration and concurrent IL-2 administration with the transferred T cells.
- Lymphocyte cultures that were selected for reactivity against melanoma antigens, including melanocyte-differentiation antigens, mediated cancer regression in some patients with metastatic melanoma. Melanoma-reactive cultures that were suitable for ACT therapy were generated from tumor-infiltrating lymphocytes that were rapidly expanded with anti-CD3 antibody.
- The generation of tumor-antigen-specific lymphocyte cultures is evolving rapidly, spurred on by the identification of tumor antigens and the T-cell receptors that recognize them.

Further improvements to ACT therapy will depend on a deeper understanding of basic immunological processes, including the role of CD4+ T cells in the antitumor inflammatory response, the ability of lymphocytes to persist in vivo and travel to tumors, and the mechanisms of ACT augmentation by previous host immunosuppression.

ACT regimen results in the in vivo expansion and enhanced activity of these cytotoxic lymphocytes. Of the 35 melanoma patients treated by adoptive cell therapy in a phase II clinical trial, 18 patients (51 %) achieved an objective response, with 3 patients exhibiting a complete response (Dudley et al. 2005). NCI has identified and characterized a number of melanoma tumor-associated antigens, including gp100 and MART-1, and has developed a lymphodepleting non-myeloablative regimen used for ACT. Transgene and the NCI have collaborated to evaluate new candidate cancer vaccines, with the objective to assess the boosting effect of the vaccination on the lymphocytes' activity. These novel vaccines were designed by Transgene using viral vectors to express melanoma antigens. Such a vaccination has already demonstrated increased in vivo clonal expansion and maintenance of adoptively transferred tumor-antigen specific cytotoxic lymphocytes in preclinical models. The NCI will conduct preclinical evaluation of the vaccines and sponsored a phase I/II trial. The adoptive transfer of in vitro generated tumor antigen-specific cytotoxic T lymphocytes (CTL) provides a promising approach to the immunotherapy of cancer. A phase I study was conducted to test the feasibility, safety, and survival of adoptively transferred Melan-A-specific CTL lines in melanoma patients and shown to induce clinical tumor-specific immune responses without major adverse effects (Mackensen et al. 2006).

Tumor infiltrating lymphocytes (TILs) already appear to offer significant patient benefit and this approach now warrants further development. Genetically engineered T cells offer a means to endow peripheral blood T cells with antitumor activity and in principle these techniques could allow the treatment of a wide range of cancers. Genetic engineering also offers the means to endow T cells with new properties and enhanced functions. Proof-of-principle trials have shown clear responses with T cell receptor-engineered T cells and this can be built on with further development (Hawkins et al. 2010).

Epstein-Barr virus (EBV) infection is associated with a heterogeneous group of tumors, including lymphoproliferative disorders, Hodgkin's disease, nasopharyngeal carcinoma and Burkett's lymphoma. As these cancers express viral antigens, they can be treated by ACT strategies relying mostly on in vitro generation and expansion of virus-specific CTL, which can be administered to patients for both prophylaxis and treatment. ACT with EBV-specific CTL is safe, well-tolerated and quite effective in the case of most immunogenic tumors, egg, post-transplant lymphoproliferative disease (Merlo et al. 2008).

### Combination of Antiangiogenic Agents with ACT

Although ACT-based immunotherapies can achieve cancer regression in animal models and in up to 70 % of patients with metastatic melanoma, it is possible that the tumor vasculature impedes the egress of tumor-specific T cells, thus hindering immunotherapy. Disruption of the proangiogenic interaction of VEGF with its receptor VEGFR-2 has been reported to "normalize" tumor vasculature, enhancing the efficacy of chemotherapeutic agents by increasing their delivery to the tumor.

Administration of an antibody against mouse VEGF synergized with ACT to enhance inhibition of established, vascularized, B16 melanoma and improved survival (Shrimali et al. 2010). Anti-VEGF antibody significantly increased infiltration of transferred cells into the tumor. Thus, normalization of tumor vasculature through disruption of the VEGF/VEGFR-2 axis can increase extravasation of adoptively transferred T cells into the tumor and improve ACT-based immunotherapy. These studies provide a rationale for the exploration of combining antiangiogenic agents with ACT for the treatment of patients with cancer.

**Genetically Targeted T Cells for Treating B Cell Malignancies**

Human T cells targeted to the B cell-specific CD19 antigen through retroviral-mediated transfer of a chimeric antigen receptor (CAR) called 19z1, have shown significant but partial in vivo antitumor efficacy in an acute lymphoblastic leukemia (ALL) model (Brentjens et al. 2007). The causes of treatment failure in this model were investigated and approaches were designed to enhance the efficacy of this adoptive strategy. Expression of the 19-28z CAR, containing the signaling domain of the CD28 receptor, enhanced systemic T-cell antitumor activity when compared with 19z1 in treated mice. T-cell injections, designed to prolong in vivo T-cell function, further improved long-term survival. Thus combined in vivo costimulation and repeated administration enhance eradication of systemic tumor by genetically targeted T cells. The finding that modifications in CAR design as well as T cell dosing enable the complete eradication of systemic disease affects the design of clinical trials using this treatment strategy. The investigators have an ongoing study using these T cells in CLL, and are planning a trial in patients with ALL. The idea is that a patient's own T cells are taken and re-educated by inserting a gene into them that will enable them to produce a receptor to recognize B cell cancers, and then they are returned to the patient where they should be able to attack and kill the tumor cells. Because the technique uses a patient's own T cells, there is little risk of compatibility issues or rejection, as there might be with human stem cell transplant. Human stem cell transplant, following radiation or chemotherapy, is currently incorporated into the treatment of several B cell malignancies.

The extensive exploitation of the antitumor effect of donor lymphocytes infused after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is limited by the risk of GVHD. To overcome this limitation, the therapeutic potential of donor lymphocytes engineered with the suicide gene thymidine kinase (TK) of HSV was investigated in patients experiencing recurrence of hematologic malignancies after allo-HSCT (Ciceri et al. 2007). The antitumor effect tightly correlated with the in vivo expansion of TK+ cells. Immunization against HSV-TK was observed in some patients but did not preclude an effective GvL. These data validate the feasibility, safety, and efficacy of TK+ cells in the context of allografting and represent the basis for a broader application of this technology. This technology is being clinically developed by MolMed.

### Genetic Engineering of Tumor Cells

Many companies have effective vaccines for stimulating killer T lymphocytes. The missing link is making good vaccines for helper T lymphocytes. That problem has been solved by Antigen Express scientists, who developed means to suppress the expression of a specific immunoregulatory protein (Ii). This protein can block antigens from stimulating T helper cells. By inhibiting this protein, a whole range of antigens from tumors can now be recognized by T helper cells, greatly boosting the immune response to cancer.

### Hybrid Cell Vaccination

The hybrid cell vaccination approach to cancer immune therapy aims at the induction of tumor-specific cytotoxic T cells and was developed for the following purposes:

- To recruit and activate T cell help for the induction of tumor-specific cytotoxic T cells
- To correct defects in co-stimulatory signaling.
- To utilize a large number of unidentified tumor-associated antigens
- For individualized therapy that can be applied instantly without long preparation.

Hybridoma technology involves selection of long-term lines on the basis of their resistance to anticancer drugs and according to specific functions desired. The fusion partners are of the same tissue origin and are controlled by similar genetic programs. The vaccines are irradiated prior to inoculation to ensure that the tumor does not grow and spread in the body.

Clinical trials of hybrid cell vaccination have been performed in patients suffering from malignant melanoma or renal cell carcinoma and cases of complete remission have been reported. The side effects seen in these trials were those of induced immune response. Hybrid cell vaccination is a feasible strategy for the treatment of cancer and is well suited for individualized therapy. Future trials will establish the criteria for selection of patients and the malignancies suitable for this therapy.

## *Personalized Peptide Cancer Vaccines*

Following identification of tumor associated antigens (TAA) in different tumor histotypes, many vaccination strategies have been investigated, including peptide-based vaccines. Results of the first decade of clinical experimentation, although demonstrating the feasibility and the good toxicity profile of this approach,

provided evidence of clinical activity only in a minority of patients despite inducing immunization in up to 50 % of them. Different approaches have been developed recently in order to induce stronger peptide-induced immune-mediated tumor growth control, possibly translating into improved clinical response rates, with specific focus on multipeptide-based anticancer vaccines (Pilla et al. 2009). This strategy offers many advantages, such as the possibility of bypassing tumor heterogeneity and selection of antigen-negative clones escaping peptide-specific immune responses, or combining HLA class I- and class II-restricted epitopes, thus eliciting both CD4- and CD8-mediated immune recognition. Notably, advances in antigen discovery technologies permit further optimization of peptide selection, in terms of identification of tumor-specific and unique TAA as well as antigens derived from different tumor microenvironment cell components. With the ultimate goal of combining peptide selection with patient-specific immunogenic profile, peptide based anticancer vaccines remain a promising personalized treatment for cancer patients, as shown by of preclinical and clinical studies. Use of personalized peptide vaccination combined with chemotherapy has been explored for cancer patients, e.g. those with breast and prostate cancers, which are described under the cancers of respective organs in a following section.

## *Current Status and Future Prospects of Personalized Cancer Vaccines*

This article has identified some of the important technologies and given examples of their application. The review of current state of technologies relevant to cancer indicates good prospects for the development of personalized cancer therapies. Some of the current clinical trials of personalized cancer vaccines are shown in Table 10.4.

Numerous other clinical trials of cancer vaccines have been conducted with a high failure rate. Reasons for failure include the following:

- The immune system is already damaged by chemotherapy in some patients and may not respond to vaccines.
- Vaccines based on a single antigen are less effective than those that to raise an immune response against a broad range of tumor antigens to minimize the chance of the tumor becoming resistant to the therapy.
- Immune response to vaccine may take a few months to evolve and tumors that grow rapidly may outpace it.
- Some cancer patients with advanced and bulky tumors are not good subjects for immunotherapy.

**Table 10.4** Clinical trials of personalized cancer vaccines

| Vaccine | Sponsor | Description/indication | Phase/status |
|---|---|---|---|
| AGS-003 autologous DC vaccine | Argos Therapeutics | Arcelis™ Personalized Immunotherapy: DCs pulsed with amplified mRNA from the patient's tumor/metastatic renal cell carcinoma | Phase II |
| BiovaxID® | Biovest International Inc | Patient-specific active immunotherapy/mantle cell lymphoma and follicular non-Hodgkin's lymphoma | Phase II/III Compassionate use in Europe |
| CVac™: dendritic cell (DC)/ auto-logous vaccine | PrimaBiomed | DCs + mannan fusion protein (adjuvant mannan, attached to a tumor cell surface protein, mucin 1)/ovarian cancer | Phase IIb |
| DC/autologous vaccines | Northwest Biotherapeutics | 1. DCVax®-Prostate/cancer of prostate<br>2. DCVax®-Brain/glioblastoma multiforme | 1. Phase III/US<br>2. Phase II/US |
| FANG™ Vaccine | Gradalis Inc | Autologous tumor cell vaccine expresses rhGM-CSF and the bifunctional RNAi effector, bi-shRNAfurin/ | Phase II |
| HER2/neu hybrid vaccine | Antigen Express | Peptide immunotherapeutic/HER2/ neu positive breast cancer | Phase II |
| Imetelstat (GRNVAC1) | Geron Corporation | Autologous DCs loaded ex vivo with telomerase reverse transcriptase/ metastatic prostate cancer | Phase II |
| MGN1601 | Mologen | Gene-modified tumor cells based on MIDGE (Minimalistic Immunogenically Defined Gene Expression) vectors/kidney cancer | Phase I |
| MyVax® | Genitope Corporation | Designed to activate a patient's immune system to identify and attack cancer cells/follicular non-Hodgkin's lymphoma | Phase III |
| Oncophage (now called Prophage, except in Russia) | Agenus Inc | Renal cancer and malignant melanoma | Phase III in US Marketed in Russia |
| OncoVAX | Vaccinogen Inc | Vaccine from the patient's own tumor cells, which are irradiated to make them non-carcinogenic/colorectal cancer | Phase III |

(continued)

**Table 10.4** (continued)

| Vaccine | Sponsor | Description/indication | Phase/status |
|---|---|---|---|
| OVAX autologous cancer cell vaccine | AVAX Technologies Inc | Haptenization, i.e. chemical modification of antigens on the cancer cell to stimulate the patient's immune system/relapsed stage III-IV ovarian cancer | Phase II |
| Personalized idiotype vaccine | Bayer Innovation GmbH | Vaccine antigen is produced in tobacco plants based on magnICON® technology | Phase I |
| Stimuvax® DC vaccine | Oncothyreon Inc/ Merck KGaA | Elicits T cell mediated immune response to cancer cells expressing the target MUC-1/ metastatic NSCLC | Phase III |
| TroVax: dendritic cell vaccine | Oxford Biomedica | Vaccinia virus Ankara is engineered to deliver the 5T4 antigen to destroy cancer cells/TRIST (TroVax Renal Immunotherapy Survival Trial) in advanced and metastatic renal cell carcinoma | Phase III |



## Personalized Radiation Therapy

Radiation therapy is the most common agent used in cancer therapy and up to 60 % of cancer patients receive it. However, physicians are unable to distinguish differences in radiosensitivity across tumors when prescribing it. Accurate prediction of human tumor response to radiation therapy and concomitant chemoradiation would be an important tool to assist the physician in making recommendations for tumor treatment. Most studies that define the molecular biomarkers for prediction of radiation response are based on the observation of gene expression using immunostaining, Northern blot or Western blot analysis of a single or several genes. The results vary among the different studies and some results are contradictory. However, these studies agree that the change in expression of the tumor-related gene affects the radiation response. A novel approach was developed to predict the radiation response of human tumor using Atlas™ human cancer 1.2 cDNA arrays to analyze the expression profile of 1,187 tumor-related genes in radiation-resistant and radiation-sensitive tissues (Hanna et al. 2001). Sixty tumor-related genes were selected as predictors of radiation response of squamous cell carcinoma of the head and neck. Using the expression intensity of these 60 tumor-related genes, in combination with cluster analysis, researchers have introduced a mathematical method that successfully predicted the radiation identity of two tumor samples.

Radiation therapy treatment comes with serious side effects in 5 % of patients. Some cases of toxicity are associated with abnormal transcriptional responses to

radiation. Screening blood for the activity level of 24 genes can identify those patients most likely to react badly to radiation (Rieger et al. 2004). This tool may help physicians to tailor treatments for individual patients. Some factors are a tip-off that a patient may have an unusually severe reaction to radiation. Patients who have autoimmune diseases such as diabetes or lupus, or who have certain rare genetic diseases need to be monitored carefully or avoid radiation altogether. Even beyond these obvious signs, some patients suffer disfiguring, disabling or extremely painful effects. These may include wounds that do not heal, skin burns so severe they require plastic surgery, or brain damage. Past attempts to identify these patients by screening the cancer cells themselves have failed. Screening blood rather than cancer cells means the test would be more accessible to patients. Patients who respond poorly to radiation might have cells that do not properly recognize or repair radiation-induced DNA damage. These cells may turn on different genes, or the same genes at different levels, compared with normal cells exposed to radiation. Knowing which patients may have severe radiation toxicity could make treatment decisions easier. For cancers of the breast or prostate, surgical options can be as effective as radiation. For other cancer patients, radiation may be the best treatment. However, patients at risk for high toxicity may also have cancers that die in response to much lower radiation doses. In such cases, radiation – though at greatly reduced doses – may still be an option. Even those patients who do not have severe radiation toxicity may also benefit from this study. If you eliminate those patients with toxicity are excluded, the remaining patients may be eligible for higher doses. If patients are treated individually rather than as averages, many could receive higher, more effective doses. Before personalized radiation treatment becomes possible, investigators must validate the 24-gene test on a larger number of patients. Then the screen needs to be commercialized to make it available to medical laboratories.

Genetic profiles of tumor response to treatment techniques available could help physicians prescribe radiation therapy customized for individual cancer patients' needs. An important finding is that a trio of proteins often present in cancer cells – NK-κB, extracellular-signal regulated kinase (ERK) and GADD45β – protect the tumor from destruction by radiotherapy and might lead to radioresistance. These proteins are co-activated by ionizing radiation in a pattern of mutually dependence to increase cell survival and defend cells against the cytotoxicity induced by ionizing radiation. Administration of drugs that block the proteins would enable irradiation of the cancer with lower radiation doses. This would not only be more effective against the cancer but also less harmful to the patient. A deeper understanding of the relationship among these protein molecules, gained through genetic testing, would be the key to a successful attack on cancer. If one can test cancer cells not for just three proteins but for thousands, the 'genetic fingerprint' such a test would provide might help the formulation of better therapies to destroy cancer.

## *Use of Radiation Sensitivity Biomarkers to Personalized Radiotherapy*

A systems-biology understanding of radiosensitivity has been used for identifying radiation-specific biomarkers (Eschrich et al. 2009). The authors used radiosensitivity modeling, as represented by the survival fraction at 2 Gy, in 48 human cancer cell lines. A linear regression algorithm was applied for integrating gene expression with biological variables, including ras status (mut/wt), tissue of origin and p53 status. The biomarker discovery platform is a network representation of the by linear regression analysis. This network of top 500 genes identified by this approach was reduced to a 10-hub network that includes c-Jun, HDAC1, RELA (p65 subunit of NFKB), PKC-beta, SUMO-1, c-Abl, STAT1, AR, CDK1, and IRF1. Nine targets associated with radiosensitization drugs were linked to the network, demonstrating clinical relevance. Furthermore, the model identified four significant radiosensitivity clusters of terms and genes. Ras was a dominant variable in the analysis, as was the tissue of origin, and their interaction with gene expression but not p53. Overrepresented biological pathways differed between clusters but included DNA repair, cell cycle, apoptosis, and metabolism. The c-Jun network hub was validated using a knockdown approach in human cell lines representing different cancers. This novel radiation-biomarker discovery platform, using a systems biology modeling approach, will play a central role in the integration of biology into clinical radiation oncology for personalizing therapy. It has been clinically-validated in rectal cancer, esophageal cancer, head and neck cancer, and breast cancer (Eschrich et al. 2012). Such a molecular assay of tumor radiosensitivity is a roadmap towards biology-based personalized radiation therapy (Torres-Roca 2012). Cvergenx, in collaboration with the Moffitt Cancer Center, is commercializing this assay.

## *Use of Imaging to Monitor Radioimmunotherapy of Non-Hodgkin Lymphoma*

Radiation dose to red marrow from $^{131}$I-rituximab is inherently underestimated by standard indirect peripheral blood counting methods. Personalized marrow dosimetry by quantitative gamma imaging more accurately predicts of hematopoietic myelotoxicity by direct measurement of the bone marrow activity concentration of $^{131}$I-rituximab. A study has measured red marrow uptake directly using serial quantitative whole-body imaging in conjunction with SPECT/CT in patients undergoing routine $^{131}$I-rituximab radioimmunotherapy of NHL (Boucek and Turner 2014). Activity clearance from whole body, measured by imaging $^{131}$I-rituximab, was significantly slower than the mean effective half-life clearance calculated from the sampling peripheral blood. Mean activity concentrations in bone marrow, measured using SPECT/CT and by blood sampling, extrapolated to the time of administration, were concordant. Neutrophil toxicity correlated with absorbed dose by SPECT/CT imaging, whereas the blood sampling method demonstrated no correlation with any parameters of hematological toxicity.

## Role of Nanobiotechnology in Personalized Management of Cancer

Role of nanobiotechnology in developing personalized approaches to the management of cancer was recognized a decade ago (Jain 2005). Nanodiagnostics has the potential to improve early diagnosis of cancer. Nanobiotechnologies will also improve detection of cancer biomarkers as a basis for devising diagnostics as well as therapeutics. Personalization of cancer therapies is based on a better understanding of the disease at the molecular level and nanotechnology will play an important role in this area (Jain 2010). Various components of personalized therapy of cancer that are relevant to nanobiotechnology are shown in Fig. 10.2.

An example of application of nanobiotechnology in improving cancer management is use of αnuβ3-targeted paramagnetic nanoparticles to noninvasively detect very small regions of angiogenesis associated with nascent melanoma tumors (Schmieder et al. 2005). Each particle is filled with thousands of molecules of the metal that is used to enhance contrast in conventional MRI scans. The surface of each particle is decorated with a substance that attaches to newly forming blood vessels that are present at tumor sites. This enables the detection of sparse biomarkers with molecular MRI in vivo when the growths are still invisible to conventional MRI. Earlier detection can potentially increase the effectiveness of treatment, particularly in case of melanoma. Another advantage of this approach is that the same nanoparticles used to detect the tumors can be used to deliver stronger doses of anticancer drugs directly to the tumor site without systemic toxicity. The nanoparticle MRI would enable physicians to more readily evaluate the effectiveness of the treatment by comparing MRI scans before and after treatment. This fulfills some of the important components of personalized cancer therapy: early detection, combination of diagnostics with therapeutics and monitoring of efficacy of therapy.

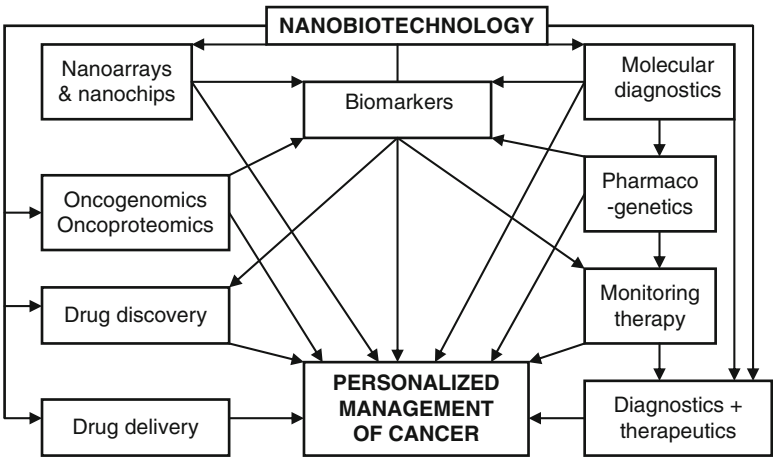


**Fig. 10.2** Role of nanobiotechnology in personalized management of cancer

Dendrimers are a novel class of 3D nanoscale, core-shell structures that can be precisely synthesized for a wide range of applications including oncology. Specialized chemistry techniques enable precise control over the physical and chemical properties of the dendrimers. They are most useful in drug delivery but can also be used for the development of new pharmaceuticals with novel activities. Polyvalent dendrimers interact simultaneously with multiple drug targets. They can be developed into novel targeted cancer therapeutics. Dendrimers can be conjugated to different biofunctional moieties such as folic acid using complementary DNA oligonucleotides to produce clustered molecules, which target cancer cells that over-express the high affinity folate receptor.

## Design of Future Personalized Cancer Therapies

A better understanding of cancer biology would enhance the design of future therapies for cancer. For example, PCR can already be used to assess the efficacy of new therapies for leukemias. Future targets for cancer therapies may include defective proto-oncogenes or the tumor suppressor genes themselves. A gene therapy strategy might be employed to correct or replace the defective gene. In cancers with multifactorial etiology, it may be possible to interrupt one or two steps in the complex pathways, thereby hindering the overall evolution of the tumor. Studies using serial analysis of gene expression have shown that tumor and normal endothelium are distinct at the molecular level, a finding that may have significant implications for the development of antiangiogenic therapies.

Mutant mice lacking cyclin D1 are entirely resistant to breast tumors induced by neu and ras, genes implicated in most human breast cancers, but are susceptible to those tumors caused by the other oncogenes c-myc and Wnt-1. Although it remains to be seen whether these findings translate to humans, the results suggest that human breast cancers caused by neu and ras could be treated with anti-cyclin D1 therapy. This would be personalized cancer therapy. Molecular profiles of breast-cancer patients could be drawn up using DNA chips or assays.

Use of emerging technologies in early clinical trials is allowing quick assessment of the efficacy of anticancer agents. Cyclacel Ltd has introduced the concept of assembling a toolkit that will allow rational drug development rather than a “trial and error” method. Identification of specific biomarker molecules in tumor tissue will permit prediction of clinical outcomes in response to drug treatment. Such biomarkers can be detected by a variety of techniques including immunohistochemistry, microarrays and qPCR. The cancer clinical trial toolkit, including biomarkers that can detect antitumor activity of anticancer agents, can guide selection of patients for specific drug treatments.

## *Personalized Therapy of Cancer Based on Cancer Stem Cells*

Cancers may rely on "cancer stem cells" that share the self-renewal feature of normal stem cells. Cancer stem cells form new tumors and may not be eliminated by current therapies. This has changed the perspective with regard to new approaches for treating cancer. Cancer stem cells are slow-dividing and inherently drug-resistant, and their eradication would be necessary for long-term success in cancer treatment. The cancer stem cell concept could be used to better tailor treatment strategies to individual patients. Most traditional anticancer agents affect primarily bulk tumor cells by disrupting their proliferation and/or survival. Even the newer 'targeted' agents, such as receptor tyrosine kinase inhibitors and some MAbs, though a considerable improvement over older agents, are still largely aimed at proliferation, survival and angiogenesis pathways that may or may not affect the stem cell population. Cancer stem cells are less likely to be killed than bulk tumor cells by these approaches. Improved methods will be required to identify, isolate and genetically profile the stem cell population in cancers from individual patients. Cancer stem cells, amplified from individual clinical specimens, should be tested for gene expression profiles and sensitivity to a battery of agents, leading to individualized decisions on selection of the best therapeutic strategies. The antineoplastic agents of the future will have to target the ancient developmental molecular pathways on which stem cells depend on for replication and survival. Thus, an improved understanding of these pathways and their roles in cancer stem cells could lead to a new generation of more selective and effective antineoplastic treatments.

## *Role of Epigenetics in Development of Personalized Cancer Therapies*

In addition to having genetic causes, cancer is also an epigenetic disease. Epigenetics refers to control of gene expression selectively without affecting the genomic DNA sequence. Epigenetic regulation of gene transcription is emerged a key biological determinant of cellular differentiation and plays a significant pathogenic role in a number of human diseases, particularly cancer. This regulation is mediated by selective, enzyme-catalyzed, covalent modification of DNA and of proteins (especially histones) that control the conformational transition between transcriptionally active and inactive states of chromatin. Disruption of the activity of disease-associated epigenetic enzymes offers a mechanism-based opportunity for pharmacologic intervention in diseases such as cancer. DNA methylation patterns undergo changes in cancer cells and represent an attractive therapeutic target because such epigenetic alterations are more readily reversible than genetic events. When used in combination with conventional chemotherapeutic agents, epigenetic-based therapies may provide a means to sensitize drug-resistant tumors to established treatments.

Epizyme Inc is focused on discovering novel, small molecule drugs that act as selective inhibitors of key epigenetic enzymes. The selective addition of methyl groups to specific sites on the histones is controlled by the action of a unique class of enzymes known as the histone methyltransferases (HMTs). Once the methyl group has been deposited on the histone site, the affected genes continue to be regulated (turned on or off) until this chemical unit is removed by other enzymes, known as histone demethylases. In a like manner, other enzyme classes can decorate DNA and histones with other chemical species and still other enzymes can remove these species to provide temporal control of gene regulation. The strategy at Epizyme Inc is to target the HMTs as a family of S-adenosyl methionine-utilizing enzymes, making full use of lessons learned from kinases as drug targets and exploiting technological platforms that allow parallel processing of multiple enzymes of similar mechanism. Two programs at Epizyme are based on epigenetics: DOT1L targeting for the treatment of mixed lineage leukemia and EZH2 targeting for the treatment of certain non-Hodgkin's lymphomas and breast cancer subtypes. Both of these programs are at subclinical stage.

Aberrant epigenetic modifications are frequently associated with distinct cancer types and have potential utility as biomarkers. The development of DNA methylation biomarkers that are predictive of a response to chemotherapy, however, is still in its infancy. Several studies have reported associations between DNA methylation biomarkers and response to chemotherapy.

## *Selective Destruction of Cancer Cells While Sparing Normal Cells*

A problem with conventional chemotherapy or radiotherapy is that damage is not limited to cancer cells but involves normal cells as well. It is easy to kill cells in vitro and many new anticancer drugs are being discovered. However, it is difficult to selectively kill cancer cells in vivo without harming normal cells. Even though some success is achieved in animal experiments, it is difficult to translate these findings into practical management of cancer patients. Strategies for selective destruction of cancer in vivo are:

- Drugs for selective disruption of cancer metabolism: sphingolipids
- Hyperbaric oxygen as adjunct to radiotherapy
- Genetically engineered bacteria for selective destruction of cancer
- Use of MAbs to selectively target anticancer agents to receptors on cancer cells
- Targeting response to transformation-induced oxidative stress
- Targeting enzymes to prevent proliferation of cancer cells: CFI-400945

### Sphingolipids

Cancer cells are sensitive to nutrient limitation because cancer cell's ability to generate ATP is compromised under these conditions. In addition, most cancer cells have defects in autophagy, the catabolic process that provides nutrients from internal sources when external nutrients are unavailable. In contrast, normal cells can adapt to the nutrient stress that kills cancer cells by becoming quiescent and catabolic. A study has shown that FTY720, a water-soluble sphingolipid drug that is effective in many animal models of cancer, selectively starves cancer cells to death by down-regulating nutrient transporter proteins (Romero Rosales et al. 2011). Consistent with a bioenergetic mechanism of action, FTY720 induced autophagy of cancer cells but normal cells were protected. AAL-149, a FTY720 analog that lacks FTY720's dose-limiting toxicity, also triggered transporter loss and killed patient-derived leukemia cells while sparing cells isolated from normal donors. Because FTY720 analogs target the metabolic profile of cancer cells rather than specific oncogenic mutations, they should be effective against several tumor types, particularly in combination with drugs that inhibit autophagy.

### Hyperbaric Oxygen as Adjunct to Radiotherapy

Hyperbaric oxygen (HBO), i.e. oxygen under higher than atmospheric pressure, is used for the treatment of several disorders. HBO has been investigated as an adjunct to radiotherapy of cancer. It is well recognized that hypoxia influences the response of cells and tissues to radiation and increases the resistance of cancer to radiotherapy requiring higher radiation doses that can normal tissues. HBO is considered to be the most effective method for counteracting tumor hypoxia for enhancing the effect of radiotherapy on cancer, but this approach has been shown to be effective in only some types of cancer, e.g. glioblastoma multiforme (Jain 2009). In spite of several studies, the controversy has not been resolved. Combination of antineoplastic agents and HBO induces dual injury to the mitochondrial respiration and cell membranes. HBO can be added to regimes combining radiotherapy with chemotherapy. Concomitant HBO enhances the effects of 5-fluorouracil on malignant tumors but no clinical trials have been done to evaluate this combination.

### Targeting Response to Transformation-Induced Oxidative Stress

Malignant transformation is often associated with enhanced cellular stress and DNA damage. Cancer cells adapt to this stress to survive, and may become dependent upon non-oncogenes that do not ordinarily perform such a vital function in normal cells. Therefore, targeting this non-oncogene dependency may result in selective death of cancer cells. A cell-based small-molecule screening and quantitative proteomics approach led to the unbiased identification of piperlongumine, a small molecule that selectively kills cancer cells but not normal cells (Raj et al. 2011). Piperlongumine increases the level of reactive oxygen species (ROS) and apoptotic

cell death in both cancer cells and normal cells engineered to have a cancer genotype, irrespective of p53 status, but it has little effect on normal cells. Significant antitumor effects were observed in mouse xenograft tumor models treated with piperlongumine, but no toxic effects were observed in normal mice. Moreover, piperlongumine inhibits the growth of spontaneous breast cancers in mice. These findings show that ability a small molecule can selectively induce apoptosis in cells that have a cancer genotype by targeting a non-oncogene dependency acquired through the expression of the cancer genotype in response to oxidative stress induced by malignant transformation.

#### Targeting Enzymes to Prevent Proliferation of Cancer Cells

CFI-400945 has been designed by a team of scientists in the Canada and China to specifically prevent proliferation of cancer cells but not damage normal cells. It targets an enzyme called PLK4, which plays a critical role in cell division, especially in cancer cells. Cells in genomically unstable cancers can have scores more chromosomes than the 46 present in normal cells, and these malignant cells rely on PLK4 to be able to continue to proliferate out of control. Targeting this enzyme would prevent survival of these cells. Animal experimental studies have been completed and FDA permission to start human clinical trials is pending with expected go ahead in the fall of 2013. Initial trial with the drug will be a study in patients with breast or ovarian cancers to determine a safe dose.

## Role of Oncoproteomics in Personalized Therapy of Cancer

Clinical proteomics is an exciting new subdiscipline of proteomics that involves the application of proteomic technologies at the bedside, and cancer, in particular, is a model disease for studying such applications. Oncoproteomics is the term used for application of proteomic technologies in oncology. Proteomic technologies are being developed to detect cancer earlier, to discover the next generation of targets and imaging biomarkers, and to tailor the therapy to the patient. Proteomic technologies will be used to design rational drugs according to the molecular profile of the cancer cell and thus facilitate the development of personalized cancer therapy. Proteomic separation and analytical techniques are uniquely capable of detecting tumor-specific alterations in proteins.

### *Cancer Tissue Proteomics*

Cancer tissue proteomics implies direct tissue profiling and use of imaging MALDI MS to provide a molecular assessment of numerous expressed proteins within a tissue sample. Analysis of thin tissue sections results in the visualization of 500–1,000

individual protein signals in the molecular weight range from 2,000 to over 200. LCM, in combination with MS, enables acquisition of protein signatures from a single cell type within a heterogeneous sample. These signals directly correlate with protein distribution within a specific region of the tissue sample. The systematic investigation of the section allows the construction of ion density maps, or specific molecular images, for virtually every signal detected in the analysis.

MALDI TOF MS can be used to generate protein spectra directly from frozen tissue sections from surgically resected cancer specimens. Profiling MALDI MS has been used to monitor alterations in protein expression associated with tumor progression and metastases. Current data suggests that MALDI MS will be superior to immunohistochemical stains and electron microscopy in identifying the site of origin for tumors currently labeled as "tumor of unknown primary". Another application in surgical pathology would be the rapid evaluation of margins of surgical excision of a tumor. Routine analysis of surgical margins by frozen section is very difficult because some cancers invade in a single cell fashion without producing a grossly identifiable mass. Sensitivity of MS enables detection of even a few tumor cells within a significantly larger portion of tissue.

The capability of MALDI MS to measure susceptibility and response to therapeutic agents in tumor and surrounding tissues is particularly useful in personalized management of cancer. The original protein profile obtained from the primary tumor can be used to influence the selection of therapeutic agents. Levels of chemotherapeutic agents can be measured directly from a tissue biopsy to assess adequacy of delivery to a particular organ site. It will also help in detecting alterations in specific molecular pathways directly modulated or indirectly affected by the anticancer agent. Finally, it could be used to monitor chemotherapy effects on the tumor.

## Role of Sequencing in Personalized Therapy of Cancer

Discoveries made through application of the human genome sequencing have already an impact on practice of oncology and have influenced the design of clinical trials for new cancer therapies. Sequencing the entire TP53 gene from various types of cancer using next-generation sequencing (NGS) with ultradeep coverage has enabled a curated mutation database for TP53 mutations and a framework for mutation database analysis (Edlund et al. 2012). Such databases are expected to play central roles in personalized medicine by providing targets for drug development and biomarkers to tailor treatments to each patient.

Comprehensive analysis of the genome sequence of individual cancers has helped uncover the specific mutations that contribute to the malignant phenotype, identify new targets for therapy, and increase the opportunities for choosing the optimal treatment for each patient, e.g. lung adenocarcinoma can now be divided into subtypes with unique genomic fingerprints associated with different outcomes and different responses to particular therapies (Collins and Hamburg 2013). Findings from the Cancer Genome Atlas demonstrate that the tissue of origin of a particular

cancer may be much less relevant to prognosis and response to therapy than the array of causative mutations (Kandoth et al. 2013). As a result, patients diagnosed with a cancer for which there are few therapeutic options may increasingly benefit from drug therapies originally aimed at other cancers that share common driver mutations. Sequencing enables the advance from current approach of targeted searches for specific mutations in individual cancers to widespread use of approaches that survey the entire genome.

In the future, research into cancer genomes will expand and cooperative global initiatives will generate full genome sequences of various cancers, yielding complete catalogues of somatic mutations in each one. These studies will reveal essentially the full repertoire of mutated cancer genes, enabling us to determine how many and what combinations of mutated cancer genes are necessary to generate an individual cancer. Sequencing will evolve from a research tool to cancer diagnostic. The rapid development of NGS technologies seems likely to be transformative. Within a few years, a complete cancer genome sequence will be obtainable for a few hundred dollars. It will important to incorporate analysis of the genome and transcriptome more widely into clinical trials in order to exploit the full clinical potential of information within the cancer genome and generating new and unexpected predictors of drug responsiveness and prognosis to enable personalized management of cancer.

Single gene testing in cancer is no longer adequate, especially with the growing numbers of targeted therapies, both currently FDA-approved and in the pipeline. If a lung tumor is not being driven by EGFR, then one immediately wants to know whether ALK is involved, and if not ALK then what about ROS, MET, PIK3CA, etc. There is a need for looking at multiple genes rather than a few select biomarkers. Therefore, sequencing of tumor at the time of diagnosis can give valuable guidance for choosing the right course of treatment, but many physicians only turn to large gene panels as a last resort, when the patient's tumor has not responded to conventional therapy or becomes metastatic.

Multiplex gene testing in cancer is still something of a controversial topic. One point of controversy is clinical. Clinicians often do not want too much information early on because it complicates their treatment planning. There are computational tools available online that can help interpret this avalanche of molecular information and give clinicians an easy-to-understand roadmap for each patient's treatment.

The other controversial issue is responsibility for payment for NGS. Genetic testing is perceived as expensive, but it is really no more expensive than the CAT scans and MRIs that are already commonly used serially throughout a course of treatment. The payers will soon come to understand that comprehensive gene testing saves money by using a patient's biomarkers to avoid unnecessary courses of expensive standard therapies that would are ineffective or even toxic to the patient. Genetic information that a physician needs is not always specific to the tumor. A targeted panel contains genes that have been carefully selected for the role they play in tumor formation, tumor treatment, or drug metabolism. Inherited G6PD gene mutation is helpful information, because it is predictive of an adverse reaction to oxidants, a class of drug that can induce a highly oxidated state. Other genes may be relevant and it is a good practice for every new cancer patient to have their tumor genome sequenced at the time of diagnosis.

## Pharmacogenomic-Based Chemotherapy

### *Whole Genome Technology to Predict Drug Resistance*

Millennium Pharmaceuticals Inc uses whole genome technologies, including gene and protein expression data, to predict the potential sensitivity or resistance of an individual patient's tumor to a single or group of drugs. The multi-center phase II trial of the proteasome inhibitor, Velcade™ (bortezomib™; PS-341) in relapsed and refractory myeloma patients has revealed significant activity in a heavily pre-treated patient population and represents the first anti-cancer agent to include pharmacogenomic (PGx) assessments during its clinical development. PGx analysis of bone marrow samples using bioinformatic algorithms indicate there are significant differences in gene expression profiles, which may predict patients likely to respond to Velcade and those likely to be refractory to treatment. These PGx analyses also show promise in their ability to detect the relevant biological pathways associated with disease progression and the mechanism(s) associated with drug resistance.

The mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) encodes for a multifunctional receptor involved in lysosomal enzyme trafficking, fetal organogenesis, cytotoxic T cell-induced apoptosis and tumor suppression. M6P/IGF2R loss of heterozygosity predicts poor therapeutic outcome in patients treated with radiotherapy alone. It also indicates that head and neck cancer patients with M6P/IGF2R allelic loss benefit most from chemotherapy added to radiotherapy.

### *Anticancer Drug Selection Based on Molecular Characteristics of Tumor*

Cancer cells have defects within their systems related to the control of the cell cycle. These modifications may, however, confer selective sensitivity to appropriately designed drug therapy. Thus, molecular defects could potentially be linked to specific drug sensitivities. Such correlations might guide the selection of drugs for therapy based on the molecular characteristics of individual tumors. An example is the treatment of breast cancer with trastuzumab (Herceptin; Genentech, USA), a humanized monoclonal antibody against the HER2 receptor. Overexpression of HER2 may occur as a somatic genetic change in breast cancer and other tumors. This correlates with poor clinical prognosis and serves as a marker for effective therapy with trastuzumab, either alone or in combination with chemotherapy. Results from randomized controlled studies show that adding trastuzumab to first-line chemotherapy seems to be beneficial in women with metastatic breast cancer that overexpresses HER2.

The molecular characterization of childhood leukemias directly affects treatment strategies. Acute lymphoblastic leukemia patients whose leukemic lymphoblasts

contain the MLL-AF4 or the BCR-ABL fusion are often candidates for allogeneic hematopoietic stem cell transplantation during first remission. Patients with acute promyelocytic leukemia who carry the PML-RAR alpha fusion respond to all-trans retinoic acid and have an excellent outcome after treatment with all-trans retinoic acid in combination with anthracyclines.

## *Testing Microsatellite-Instability for Response to Chemotherapy*

Microsatellites are stretches of DNA in which a short motif (usually one to five nucleotides long) is repeated several times. Microsatellite instability occurs when a germ-line microsatellite allele has gained or lost repeat units and has thus undergone a somatic change in length. Because this type of alteration can be detected only if many cells are affected by the same change, it is an indicator of the clonal expansion, which is typical of a neoplasm.

To test for microsatellite instability, DNA from the tumor and from normal tissue (blood, a buccal smear, or normal colonic mucosa) is tested by genotyping fluorescently labeled PCR products with the use of an automated sequencer. A panel of five microsatellite markers is usually adequate with microsatellite instability in two or more of them indicate a positive result. Such tests could help physicians determine a patient's prognosis and serve as a guide to therapy.

Fluorouracil-based adjuvant chemotherapy benefits patients with stage II or stage III colon cancer with microsatellite-stable tumors or tumors exhibiting low-frequency microsatellite instability but not those with tumors exhibiting high-frequency microsatellite instability. Although the results in vitro studies suggest that fluorouracil-based adjuvant chemotherapy is not beneficial in patients with colon cancer exhibiting high-frequency microsatellite instability, other drugs, such as the topoisomerase-I inhibitor camptothecin, have been shown to kill mismatch-repair-deficient cancer cells exhibiting high-frequency microsatellite instability. Therefore, it would be important to conduct molecular analyses of specimens from recent clinical trials of non-fluorouracil-based chemotherapies and to ensure that future trials include analyses of molecular pathways. In this retrospective analysis, the finding that fluorouracil-based adjuvant chemotherapy does not significantly increase, and may potentially decrease, overall and disease-free survival among patients with tumors exhibiting high-frequency microsatellite instability raises several provocative issues regarding postoperative management of stage II and stage III colon cancer. Currently available evidence is not strong enough for decision-making in clinical practice. However, these findings, if confirmed by other analyses of previous, well-designed clinical trials or by future prospective, randomized, controlled studies, indicate that microsatellite-instability testing should be conducted routinely and the results used to direct rational adjuvant chemotherapy in colon cancer.

## Pharmacogenetics of Cancer Chemotherapy

Present clinical algorithms assign adjuvant chemotherapy according to prognosis, but clinical decision-making would be greatly improved if reliable predictive markers were available to identify which subsets of patients benefit most from treatment. Another problem is that unpredictable efficacy and high levels of systemic toxicity are common in cancer chemotherapy. Genetic variability in drug-metabolizing enzymes and signaling pathways affects chemotherapy-related toxicity and treatment outcome in cancer. Pharmacogenetics, therefore, is particularly appealing for oncology. Cytotoxicity to chemotherapy agents, 5-fluorouracil and docetaxel, which have distinct mechanisms of action, are heritable traits varying with dose. Polymorphisms in thymidylate synthase (TS), MTHFR, and FCGR3A, as well as the polymorphic DNA repair genes XPD and XRCC1, influence response to chemotherapy and survival outcomes.

In breast and colorectal cancer, polymorphisms in metabolic enzymes involved in tamoxifen and irinotecan therapies has led the FDA to address genetic factors relevant to patient consideration of treatment with these compounds. Tamoxifen therapeutic failure in breast cancer has been associated with reduced CYP2D6 activity due to inefficient activation of tamoxifen. Irinotecan toxicity in colorectal cancer is more common in patients with reduced-activity UGT1A alleles, resulting in excessive exposure to the potent SN-38 metabolite. In colorectal and lung cancers, somatic mutations in the EGFR and downstream signaling molecules have been associated with the therapeutic outcome of EGFR-directed therapies. Current advances in single gene – UGT1A1, CYP2D6, EGFR, and KRAS – or multigene analysis, contribute to optimizing breast, colorectal, and lung cancer therapy highlighting how pharmacogenetics has helped in personalized decision-making for patient management (Snozek et al. 2009).

### *CYP 1A2*

The enzyme product of CYP1A2 is involved in a number of environmental carcinogens as well as anticancer drugs such as tamoxifen and drugs used for preventing nausea associated with chemotherapy such asondasetron. Other therapeutic drugs metabolized by CYP1A2 include acetaminophen, amitriptyline, clomipramine, clozapine, diazepam, methadone, propranolol, and tacrine. This shows the complexity of situations that can be encountered with co-administration of drugs in cancer patients in the presence of carcinogens. There are marked interindividual differences in capacity for CYP1A2 induction, which correlate with genetic polymorphisms termed CYP1A2F. Identification of individuals who have different capacities for induction of CYP1A2 may be an indicator of increased risk of drug interactions or drug toxicity when treated with drugs metabolized by CYP1A2. Genotyping of cancer patients prior to treatment may help to individualize treatment to avoid adverse reactions and increase the effectiveness of therapy.

## *Thiopurine Methyltransferase*

Polymorphisms in the thiopurine methyltransferase (TPMT) gene have been convincingly associated with the therapeutic efficacy and toxicity of thiopurine chemotherapeutic agents: 6-mercaptopurine and 6-thioguanine. TMPT-deficient patients are at high risk of developing severe hematopoietic toxicity if treated with conventional doses of thiopurines. Insights gained from studies of the TPMT polymorphism illustrate the potential of pharmacogenomics to optimize cancer therapy by avoiding toxic side effects in genetically distinct subgroups of patients.

Genetic polymorphism at this gene locus is associated with difficulty in achieving an effective dose of chemotherapeutic drugs in children with leukemia. Children with inherited TPMT deficiency exhibit severe hematopoietic toxicity when exposed to drugs such as 6-mercaptopurine, whereas those with a high activity form of the enzyme require high doses of the drug to achieve any clinical benefit. The TPMT polymorphism is relatively rare, with only about 1 % of the white population being homozygous for it, but, since these individuals show exaggerated toxic responses to normal doses of thiopurine, TPMT phenotype may be an important factor in the successful treatment of childhood leukemia. About 10 % of children with leukemia are intolerant to 6-mercaptopurine because of genetic defects in mercaptopurine inactivation by TPMT. Some centers already provide a diagnostic phenotyping service to guide the clinical use of 6-mercaptopurine.

A pharmacogenomic test, developed at St. Jude Children's Research Hospital, enables physicians to predetermine patients' TPMT activity levels based on whether or not they have inherited the alleles associated with TPMT deficiency. The test classifies patients according to normal, intermediate, and deficient levels of TPMT activity. Concordance between genotype and phenotype approaches 100 %. Patients classified as normal in activity – about 90 % of whites and blacks – are treated with conventional doses. Lower doses are tailored to avoid toxicity in deficient and intermediate patients, who represent about 10 % of each of these populations. The TPMT genetic test is well recognized in the effective clinical management of patients with acute lymphoblastic leukemia. Adjusting the dose of 6-mercaptopurine by a 10- to 15-fold decrease compared with conventional doses makes thiopurine as tolerable and effective in TPMT-deficient patients as it is in patients with normal activity levels.

## *Dihydropyrimidine Dehydrogenase*

Dihydropyrimidine dehydrogenase (DPD) is responsible for 80 % of the degradation of 5-fluorouracil (5-FU), a commonly used anticancer therapy. 5-FU is a prodrug that requires activation to 5-fluoro-2-deoxyuridine monophosphate (5-FdUMP) to exert antitumor activity. 5-FdUMP inhibits tumor cell replication via inhibition of thymidine synthase, an enzyme that is required for the synthesis of pyrimidine and

this inhibition slows down the tumor growth. Intravenously administered 5-FU is inactivated by dihydropyrimidine (DPD), an enzyme that exhibits wide variations among individuals. Patients with low DPD accumulate excessive 5-FdUMP, which causes severe gastrointestinal and neurological toxicities.

Approximately 3 % of Caucasians have a deficiency of the enzyme DPD. Patients with a DPD deficiency who receive 5-FU have a prolonged half-life of the active compound and may experience life-threatening and even fatal toxicities including neurotoxicity and hematopoietic toxicity. On the other hand, overexpression of DPD in tumor tissues is associated with 5-fluorouracil resistance, as determined by gene expression profiling. This suggests the need to individualize therapy to avoid enhanced toxicity. Cimetidine is an inhibitor of DPD and, therefore, concomitant use of cimetidine with 5-FU can result in similar toxicities. There are numerous mutations that may occur, making the assay difficult to perform and standardize.

## *UGT1A1 Test as Guide to Irinotecan Therapy*

Although most patients tolerate the chemotherapeutic agent irinotecan (Campostar®) for colorectal cancer quite well, some patients are genetically predisposed to severe side effects. Earlier studies with the irinotecan demonstrated that the highly variable toxicity was related to variability in the drug's metabolism. It was subsequently found that patients with two copies of one version of the UGT1A1 gene had few side effects at the standard dosage. Patients with only one copy of this version had more difficulty, and patients with two copies of the alternative version were at high risk for severe side effects. Therefore, relying on one standard dose meant that some of those patients received subtherapeutic doses of irinotecan and others received more than they could manage. UGT1A1 test was developed as a companion diagnostic to irinotecan therapy.

Third Wave Technologies had received FDA approval for its UGT1A1 test kit in 2005, but initially the test was only available only to patients enrolled in studies at the University of Chicago. Later on, the Mayo Clinic (Rochester, MN) licensed this test from the University of Chicago. Through this licensing agreement, Mayo Clinic's reference laboratory, Mayo Medical Laboratories (MML), make the test available to patients nationwide. Dosing based on the UGT1A1 test has the dual advantage of reducing side effects and increasing benefit of this important drug. Because of this study, the FDA required amendment of the package insert for Camptosar® (Pfizer) to include a warning that patients with a particular UGT1A1 genotype should receive a lower starting dose. The UGT1A1 test enables the physician to know in advance which patients are at risk. Those patients could be given reduced doses of irinotecan or other chemotherapy drugs. Genotyping results of UGT1A1 gene appear to predict severe adverse reactions more straightforward than the pharmacokinetic parameters or the phenotypes of the enzymatic activity.

A case-control study of Japanese cancer patients revealed that those with the variant UGT1A1 alleles were at significantly higher risk of severe adverse reactions to irinotecan (Ando et al. 2005). However, findings of subsequent irinotecan pharmacogenetic studies have been inconsistent. In a metaanalysis, data presented in nine studies that included a total of 10 sets of patients was reviewed for assessment of the association of irinotecan dose with the risk of irinotecan-related hematologic toxicities for patients with a UGT1A1*28/*28 genotype (Hoskins et al. 2007). The risk of toxicity was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype at both medium and high doses of irinotecan, but risk was similar at lower doses. The risk of experiencing irinotecan-induced hematologic toxicity for patients with a UGT1A1*28/*28 genotype thus appears to be a function of the dose of irinotecan administered.

## Role of Computational Models in Personalized Anticancer Therapy

### *A Computational Model of Kinetically Tailored Treatment*

Histological characteristics of a tumor are not a reliable indicator the natural history. Mechanism-based framework using cDNA arrays and computational models have promise in improving diagnosis and prediction, and thereby making tailored therapy possible. Treatment strategies may be tailored to individuals based on tumor cell kinetics. Computational models of kinetically tailored treatment have been developed to predict drug combinations, doses, and schedules likely to be effective in reducing tumor size and prolonging patient life. Such models incorporate intratumor heterogeneity as well as evolution of drug resistance, apoptotic rates, and cell division rates. These models may predict how combination chemotherapy of cell-cycle phase-specific, phase-non-specific, and cytostatic drugs affect tumor growth and evolution. Additional tests of the model are needed in which physicians collect information on apoptotic and proliferative indices, cell-cycle times, and drug resistance from biopsies of each individual's tumor. Computational models may become important tools to help optimize and tailor cancer treatments. Ideal characteristics of anticancer drug development suitable for personalized approach are:

- Designed to inhibit specific biologic pathways involved in oncogenesis
- Mechanistic specificity rather than organ/tissue selectivity
- Should fit with initiatives in individualized therapy: cDNA arrays and computational models
- Synergistic with other chemotherapeutic agents
- Prevent or delay the emergence of resistance
- Transform cancer into a chronic disease by delaying time-to-progression

### *Mathematical Modeling of Tumor Microenvironments*

The environment of a tumor is crucial determining factor in its development. A multiscale mathematical model of cancer invasion, which considers cellular and microenvironmental factors simultaneously and interactively, which can forecast how tumors grow and invade tissue (Anderson et al. 2006). The model simulations predict that harsh tumor microenvironment conditions (e.g., hypoxia, heterogeneous extracellular matrix) exert a dramatic selective force on the tumor, which grows as an invasive mass with fingering margins, dominated by a few clones with aggressive traits. In contrast, mild microenvironment conditions (e.g. normoxia, homogeneous matrix) allow clones with similar aggressive traits to coexist with less aggressive phenotypes in a heterogeneous tumor mass with smooth, noninvasive margins. Thus, the genetic make-up of a cancer cell may realize its invasive potential through a clonal evolution process driven by definable microenvironmental selective forces. The model shows a clear relationship between the shape of a cancer tumor and how aggressive it is. Aggressive tumors tend to assume a spidery shape in the model, while more benign growths are generally more spherical in shape. The findings would influence decision on how certain cancers are treated, by considering the environment around the tumor to be a contributory factor in how aggressive the cancer. Most of the current treatments are focused on making the tissue environment as harsh as possible for the tumor in the hope of destroying it. But this could allow the most aggressive cancer cells to dominate any residual tumor left after treatment and develop resistance to treatment. Moreover, these aggressive cells tend to be the more invasive resulting in an increased chance of metastasis. With use of the tools of mathematical modeling and computer simulation, cancer treatment will no longer be a trial and error game. With mathematics-driven oncology research, it will be possible to determine which drugs will work at which stage. In the future this research could help personalize treatment in a patient specific manner.

### *Modeling Signaling Pathways to Reposition Anticancer Drugs*

Computational modeling has been to derive specific downstream signaling pathways, cancer signaling bridges (CSB), which reveal previously unknown target-disease connections and have the potential for systematic as well as fast-tracked drug repositioning based on available patient gene expression data (Zhao et al. 2013). This model was applied to reposition known or shelved drugs for brain, lung, and bone metastases of breast cancer with the hypothesis that cancer subtypes have their own specific signaling mechanisms. To test the hypothesis, the authors addressed specific CSBs for each metastasis that satisfy (i) CSB proteins are activated by the maximal number of enriched signaling pathways specific to a given metastasis, and (ii) CSB proteins are involved in the most differential expressed

coding genes specific to each breast cancer metastasis. The identified signaling networks for the three types of breast cancer metastases contain 31, 15, and 18 proteins and were used to reposition drug candidates for the brain, lung, and bone metastases. Both in vitro and in vivo preclinical experiments were conducted as well as analysis on patient tumor specimens to evaluate the targets and repositioned drugs. FDA-approved drugs, sunitinib and dasatinib, were found to inhibit brain metastases derived from breast cancer.

## Therapy Resistance in Cancer

Human cancers are mostly found to be resistant to therapy at the time of drug presentation (primary responses), tumors being intrinsically drug resistant (innate or de novo drug resistance). Only a few become resistant after an initial response (acquired responses), the tumors developing resistance to chemotherapy during treatment (acquired drug resistance). In the latter group, a tumor cell may express drug resistance by combining several distinct mechanisms induced by its exposure to various drugs. In the former group, however, this is unlikely to be the case.

### *Mechanism of Therapy Resistance in Cancer*

One explanation of development of resistance is that when cells become cancerous, they also become 100 times more likely to genetically mutate than regular cells. Mutations protect cancer cells from therapeutics designed to target a particular oncogene. A single tumor may have cells with many different types of oncogenes and drug-resistant genes. Molecular diagnostics will help determine the stage and malignancy of a tumor by testing the number of its mutations. The more mutations, the further along the tumor may be in its development to malignancy or metastasis. Resistance to drugs that shut down oncoprotein-driven pathways can occur because of compensatory changes in connecting pathways. Loss of expression of MED12, which acts in the TGFβ signaling pathway, may mediate resistance to gefitinib and vemurafenib (Rosell 2013).

Pharmacogenetics and pharmacogenomics studies of the relationship between individual variations and drug response rates reveal that genetic polymorphisms of specific genes is associated with clinical outcomes in patients treated through chemotherapy, and amplification of genes encoding drug targets or transporters alters the sensitivity of cancer cells to a particular chemotherapy. Loss of heterozygosity (LOH) at specific regions of chromosomes has been identified in specific cancers but its effect on treatment outcome remains controversial.

### Role of Splice Variants in Resistance to Cancer Therapy

Alternative splicing is important for increasing the diversity of the cellular proteome, and is a process frequently deregulated during cancer development and progression. In cancer cells, diverse splicing alterations have been identified that eliminate protein domains or enzymatic activities required for efficacy of cancer therapies, promote gain of novel signaling functions that circumvent cancer therapies, and uncouple signaling pathways from upstream regulatory points that are blocked by cancer therapies. The mechanisms underlying these splicing changes range from stable alterations in gene sequence/structure to deregulation of splicing regulatory factors. An understanding of these processes is leading to the development of novel strategies for therapy re-sensitization (Dehm 2013).

### Expression of P-Glycoprotein Gene by Tumor

The mechanism underlying multidrug resistance is a cellular pump called P-glycoprotein, which normally protects cells from toxic substances by actively exporting the offending compounds. In cancer, abundant P-glycoprotein gene (MDR-1) expression by a tumor has been implicated as one of the major reasons that cancer cells develop resistance to chemotherapy. Overexpression of MDR-1 in tumors has been associated with resistance to adriamycin, paclitaxel, and many more anticancer drugs. A simple DNA test has been devised by Epidauros Biotechnologie AG that enables a physician to predict drug uptake from the beginning of therapy of cancer and avoid the trial and error approach. This test for detection of gene polymorphisms is based on the knowledge that MDR-1 has 15 polymorphisms of which only one correlates with poor drug uptake.

### Overexpression of Multidrug Resistance Gene

Approximately 75 % of cancer patients are intrinsically unresponsive or develop resistance to anticancer drugs. The mechanism underlying multidrug resistance (MDR) is a cellular pump called P-glycoprotein. Under normal circumstances, P-glycoprotein protects cells from toxic substances by actively exporting the offending compounds. In cancer, abundant P-glycoprotein gene (MDR-1) expression by a tumor has been implicated as one of the major reasons that cancer cells develop resistance to chemotherapy. Overexpression of MDR-1 in tumors has been associated with resistance to adriamycin, paclitaxel, and many more anticancer drugs. A simple DNA test has been devised by Epidauros Biotechnology that enables a physician to predict drug uptake from the beginning of therapy of cancer and avoid the trial and error approach. This test for detection of gene polymorphisms is based on the knowledge that MDR-1 has 15 polymorphisms of which only one correlates with poor drug uptake. Once detected, management of drug-resistance is still

problematic as there is no ideal remedy for it. One compound, OC 144-093 (Ontogen Corporation, Carlsbad, California) has passed phase I single blind, placebo-controlled trials. This compound is orally active, non-toxic and does not interact with paclitaxel.

### P53 Mutations

The function of the human p53 gene, sometimes associated with drug-resistance, remains only partially understood. In response to cellular stresses such as DNA damage or oncogene activation, p53 acts as a tumor suppressor by blocking cell division or inducing cell suicide through apoptosis. If p53 is mutated or otherwise inactivated, a cell can accumulate further mutations that lead to tumor formation. Furthermore, tumor cells with mutant p53 are typically unable to invoke apoptosis in response to DNA damage, rendering such tumors resistant to traditional chemotherapy and radiation therapy.

## *Detection of Drug Resistance*

### Anaplastic Lymphoma Kinase

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase of the insulin receptor superfamily. Translocations (fusions) of ALK have an established pathogenic role in more than 250,000 new cancer diagnoses in the US each year. Detection of ALK mutations has been considered increasingly important in the diagnosis and therapy selection for many types of cancer, including NSCLC, diffuse large B-cell lymphoma, anaplastic large cell lymphoma, neuroblastoma and inflammatory myofibroblastic tumors. Because of the potential for ALK-inhibitor therapies to treat so many cancers, there are several ALK inhibitors currently in development by pharmaceutical firms. ALK Assays (Insight Genetics) are based on the need for better methods of not only detecting activating ALK fusions and upregulation across many cancer types and but also monitoring for resistance mutations that arise in response to ALK-inhibitor therapy. Insight ALK Screen assay provides quick, accurate detection of any ALK fusion. Insight ALK Resistance Monitoring assays assist in monitoring patients for ALK resistance mutations.

### Metabolic Profiling of Cancer

Acquired resistance to imatinib mesylate is an increasing and continued challenge in the treatment of BCR-ABL tyrosine kinase positive leukemias as well as gastrointestinal stromal tumors. Stable isotope-based dynamic metabolic profiling (SIDMAP) studies conducted in parallel with the development and clinical testing

of imatinib revealed that this targeted drug is most effective in controlling glucose transport, direct glucose oxidation for RNA ribose synthesis in the pentose cycle, as well as de novo long-chain fatty acid synthesis. Thus imatinib deprives transformed cells of the key substrate of macromolecule synthesis, malignant cell proliferation, and growth. Tracer-based MRS studies revealed a restitution of mitochondrial glucose metabolism and an increased energy state by reversing the Warburg effect, consistent with a subsequent decrease in anaerobic glycolysis. Recent in vitro SIDMAP studies that involved myeloid cells isolated from patients who developed resistance against imatinib indicated that non-oxidative ribose synthesis from glucose and decreased mitochondrial glucose oxidation are reliable metabolic signatures of drug resistance and disease progression. There is also evidence that imatinib-resistant cells utilize alternate substrates for macromolecule synthesis to overcome limited glucose transport controlled by imatinib. The main clinical implications involve early detection of imatinib resistance and the identification of new metabolic enzyme targets with the potential of overcoming drug resistance downstream of the various genetic and BCR-ABL-expression derived mechanisms. Metabolic profiling is an essential tool used to predict, clinically detect, and treat targeted drug resistance. This need arises from the fact that targeted drugs are narrowly conceived against genes and proteins but the metabolic network is inherently complex and flexible to activate alternative macromolecule synthesis pathways that targeted drugs fail to control.

## *Management of Drug Resistance in Cancer*

### Chemogenomic Approach to Drug Resistance

Resistance to anticancer drugs represents a serious obstacle to successful cancer treatment. Genome-wide studies correlating drug response phenotypes with large DNA/tissue microarray and proteomic datasets have been performed to identify the genes and proteins involved in chemosensitivity or drug resistance. The goal is to identify a set of chemosensitivity and/or resistance genes for each drug that are predictive of treatment response. Therefore, validated pharmacogenomic biomarkers offer the potential for the selection of optimal treatment regimens for individual patients and for identifying novel therapeutic targets to overcome drug resistance.

Approximately 10 % of patients with chemotherapy-resistant bowel cancer that has spread to other parts of the body respond to treatment with MAbs – cetuximab or panitumumab. These drugs target the EGFR. However, not every CRC patient responds well to this treatment. An understanding the molecular basis of clinical sensitivity or resistance to anti-EGFR agents might identify patients who are likely to benefit from treatment with these MAbs. Those not likely to respond to MAb treatment should be spared the expense and potential adverse effects.

KRAS mutations are associated with anti-EGFR resistance. In patients with wild-type KRAS, the presence of BRAF mutation or PIK3CA mutations is

associated with lower disease control rate, shorter progression-free survival, and shorter overall survival. MET overexpression, in addition to BRAF and PIK3CA mutations, is a predictive biomarker for responsiveness to anti-EGFR MAbs in mCRC patients with wild-type KRAS (Kishiki et al. 2014). In patients with KRAS mutations, those with high levels of hepatocyte growth factor (HGF) or epiregulin (EREG) have shorter progression-free survival and overall survival compared with those with low levels of HGF or EREG (Takahashi et al. 2014). One study has explored miRNAs as biomarkers of response to anti-EGFR MAbs and found that MiR-99a/Let-7c/miR-125b signature may improve the selection of patients with KRAS wild-type mCRC as good candidates for this therapy (Cappuzzo et al. 2014).

### Determination of Chemotherapy Response by Topoisomerase Levels

Topoisomerase poisons are chemotherapeutic agents that are used extensively for treating human malignancies. These drugs can be highly effective, yet tumors are frequently refractory to treatment or become resistant upon tumor relapse. Top2A expression levels are major determinants of response to the topoisomerase-2 poison doxorubicin and suppression of Top2A produces resistance to doxorubicin. Suppression of Top1 produces resistance to the topoisomerase 1 poison camptothecin but hypersensitizes cancer cells to doxorubicin. Lymphomas relapsing after treatment display spontaneous changes in topoisomerase levels as predicted by in vitro gene knockdown studies using RNAi screens in animal models of cancer. Thus pooled shRNA screens can be used for identifying genetic determinants (biomarkers) of chemotherapy response and improve the effectiveness of topoisomerase poisons in the clinic (Burgess et al. 2008).

### Management of Drug Resistance in Leukemia

Imatinib mesylate (Novartis' Gleevec), approved in 2001, causes remission in patients with chronic myeloid leukemia (CML). Despite these positive response rates, a subset of patients do not respond to Gleevec therapy fully or at all, and approximately 4–5 % of successfully treated patients annually develop resistance to Gleevec during therapy with a return of their disease manifestations. The molecular hallmark of CML is a mutation known as BCR-ABL. This mutation is the specific target for Gleevec and is found in 95 % of patients with CML. Secondary mutations in the ABL portion of the gene correlate with treatment failure or relapse in most patients on Gleevec therapy. Genzyme has licensed exclusive worldwide diagnostic rights from the University of California (Los Angeles, CA) Jonsson Cancer Center to its discovery of gene mutations believed to be associated with drug resistance to Gleevec. Genzyme will be the first company to develop and market a diagnostic test to detect a significant portion of these secondary BCR-ABL mutations and monitor resistance in CML patients prior to and during treatment with Gleevec. Results from such a test may assist physicians in predicting patient relapse before it happens and making appropriate adjustments in treatment.

A novel pyrido[2,3-d]pyrimidine derivative, PD180970, has been shown to potently inhibit Bcr-Abl and induce apoptosis in Bcr-Abl-expressing leukemic cells in patients who develop a resistance to Gleevec. Developing additional Abl kinase inhibitors would be useful as a treatment strategy for chronic myelogenous leukemia. The key to curing more CML patients is to provide customized treatment for each individual, based on the particular molecular mutation that causes their resistance to Gleevec. Leukemia cells from patients with advanced CML should be profiled and the appropriate inhibitor or combination of inhibitors selected for treatment. This approach is similar to the method that has been used to treat HIV drug resistance. Treatment would be individualized for each patient, by combining specific inhibitors in an 'inhibitor cocktail' that would be able to combat various Bcr-Abl isoforms. 'The paradigm is to understand the genetic abnormality that drives the growth and survival of cancer, and tailor a treatment to reverse this genetic defect.

Development of B cell receptor antagonists has been a therapeutic advance in chronic lymphocytic leukemia (CLL). Although B cell receptor ligation in normal cells induces proliferation, apoptosis, or anergy, pathway dysregulation in CLL results in the propagation of proliferative and prosurvival signals. Several agents targeting the B cell receptor pathway are in development, including the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib. A sequencing study showed that acquired resistance to ibrutinib is due at least in part to recurrent mutations in BTK and PLCγ2 (Woyach et al. 2014). C481S mutation in BTK confers resistance to ibrutinib by preventing irreversible drug binding. The S707Y, R665W, and L845F mutations in PLCγ2 are all potentially gain-of-function mutations that allow B cell receptor-mediated activation that is independent of BTK. In October 2014, the European Commission approved ibrutinib (Imbruvica™) once daily capsules for the treatment of adult patients with relapsed or refractory MCL or CLL who have received at least one prior therapy, or as first line therapy in the presence of 17p deletion or TP53 mutation in patients unsuitable for chemo-immunotherapy. In patients without B cell receptor pathway mutations, resistance may be mediated through mutations in other coding genes providing alternative survival signals that are not inhibited by ibrutinib or through noncoding RNA, epigenetic activation or silencing, or selective gene amplification. Other mutations may act in combination with BTK or PLCγ2 mutations to drive resistance. Knowledge of downstream mediators of resistance may lead to the development of rational combinations to prevent or treat resistant disease.

### Resistance to Vaccines in Cancer Recurrence After Surgery

Of the >700,000 patients who undergo cancer surgery in the US each year, >40 % develop recurrences that have a poor outcome. Recurrent tumor cells have few phenotypical differences from those in tumors prior to surgery. An alternative explanation proposed for the resistance of recurrent tumors is that surgery promotes inhibitory factors that allow lingering immunosuppressive cells to repopulate small

pockets of residual disease quickly (Predina et al. 2013). These authors found that recurrent tumors and draining lymph nodes are infiltrated with M2 macrophages and CD4+ Foxp3+ regulatory T cells. This complex network of immunosuppression in the tumor microenvironment explains the resistance of tumor recurrences to conventional cancer vaccines despite small tumor size, an intact antitumor immune response, and unaltered cancer cells. Therapeutic strategies coupling anticancer agents with inhibition of immunosuppressive cells potentially could impact the outcomes in these patients.

### Systems Biology Approach to Drug-Resistant Cancer

Resistance to targeted cancer therapies such as trastuzumab may occur not only because of insufficient expression of HER2 receptor but also because of the overriding activation states of cell signaling pathways. Systems biology approaches lend themselves to rapid in silico testing of factors, which may confer resistance to targeted therapies. A new kinetic model could be interrogated to predict resistance to receptor tyrosine kinase (RTK) inhibitor therapies and directly test predictions in vitro and in clinical samples (Faratian et al. 2009). The mathematical model includes RTK inhibitor antibody binding, HER2/HER3 dimerization and inhibition, AKT/mitogen-activated protein kinase cross-talk, and the regulatory properties of PTEN. The model includes parameters based on quantitative phosphoprotein expression data from cancer cell lines using reverse-phase protein microarrays. Quantitative PTEN protein expression was found to be the key determinant of resistance to anti-HER2 therapy in silico, which was predictive of virtual experiments in vitro using the PTEN inhibitor bp(V). When measured in cancer cell lines, PTEN expression predicts sensitivity to anti-HER2 therapy; furthermore, this quantitative measurement is more predictive of response than other pathway components taken in isolation and when tested by multivariate analysis in a cohort of 122 breast cancers treated with trastuzumab. Thus a systems biology approach has been successfully used to stratify patients for personalized therapy in cancer and is further compelling evidence that PTEN, appropriately measured in the clinical setting, refines clinical decision making in patients treated with anti-HER2 therapies.

## Personalized Therapy of Cancer Metastases

Metastasis is the major cause of mortality in cancer. Primary tumors tend to metastasize to defined subsets of secondary organs, but the underlying mechanisms are not well understood. A microfluidic 3D in vitro model was developed to analyze organ-specific human breast cancer cell extravasation into bone- and muscle-mimicking microenvironments through a microvascular network (Jeon et al. 2015). Extravasation rates and microvasculature permeabilities in the bone-mimicking

microenvironment were significantly different from those in myoblast containing matrices. Blocking breast cancer cell A3 adenosine receptors resulted in higher extravasation rates of cancer cells into the myoblast-containing matrices compared with untreated cells, suggesting a role for adenosine in reducing extravasation. These results show the usefulness of microfluidic 3D model as a drug screening platform and a promising tool for investigating specific molecular pathways involved in cancer biology, with potential applications to personalized medicine.

## Personalized Management of Cancers of Various Organs

### *Personalized Management of Brain Tumors*

Brain tumors can be benign or malignant, with the latter being more frequent. Most of the discussion in this section is about glioblastoma multiforme (GBM), which is the most malignant and most frequent brain tumor and is currently incurable with a median survival of <2 years after diagnosis and treatment. Worldwide ~175,000 cases occur annually of which 17,000 are diagnosed in the US. Several innovative treatments are being developed but the mainstays of conventional treatment are chemotherapy and radiation. Chemotherapy gives inconsistent results in terms of prolongation of survival. GBM is a complex, heterogeneous disease, which makes it unlikely that a uniform approach would be suitable for all patients. There is need for the development of personalized treatment modalities to address the heterogeneity of this complex tumor phenotype.

#### Aptamers for Selective Targeting of Tumor Initiating Cells in GBM

GBM displays cellular hierarchy with self-renewing tumor-initiating cells (TIC), also known as cancer stem cells (CSCs), at the apex. Although the TIC hypothesis remains controversial and the functional assays to define the TIC phenotype are evolving, it has been shown that TICs may contribute to angiogenesis, spread of tumor, and resistance to therapy. However, identification of TICs by use of biomarkers characterized in normal stem cells has an inherent limitation to selectively identify TICs. A study adopted Cell-Systematic Evolution of Ligands by Exponential Enrichment (Cell-SELEX) to identify aptamers that specifically bind to TICs in GBM but not to human neural stem cells (Kim et al. 2013). These aptamers select and internalize into GBM cells that self-renew, proliferate, and initiate tumors. As aptamers can be modified to deliver payloads, aptamers may represent novel agents that could selectively target or facilitate imaging of TICs, which may be important for improving therapeutic outcomes in individual patients.

### Bioinformatic Approach to Personalizing Treatment of GBM

An example of bioinformatic approach to personalize treatment of GBM, is IBM's collaboration with New York Genome Center (NYGC) to use its Watson supercomputer to make sense of vast amounts of genetic sequencing data and medical information for identifying personalized treatments for cancer patients. The partners will test a Watson prototype designed specifically for genomic research to help oncologists deliver unique, customized cancer treatments according to an individual patient's DNA. A search of alternative treatments for glioblastoma multiforme will test this approach.

GBM patients at the NYGC's member institutions will be selected for the Watson study. Each patient's tumors will be sequenced at the Genome Center on Illumina servers running algorithms in the IBM SoftLayer cloud. Biopsies are conducted on patients, and both normal and cancer cells are sequenced by the NYGC's servers. The sequencing normally takes 10–12 days because of the intricacy of the task; regular cells have to be sequenced ~30 times and cancer cells 30–50 times. In the slow-but-groundbreaking process, algorithms developed through years of public and private sector research create perfect representations of the patients' cells in bits and bytes.

In the next stage, which takes a few weeks, the raw sequences for healthy and cancerous cells are extrapolated and put through heuristic algorithms to figure out what healthy and cancerous cells look like in each patient. This information is used to create variant call files – raw info files used by the NYGC's software to store gene sequence variations. These files are what Watson uses to find novel cancer treatments. Each variant file can contain between 20,000 to 1 million potential mutations. Among them is a driver mutation that primarily fuels the cancer, and passenger mutations that have much less effect. Watson combines findings from the NYGC's programs with automated queries of a massive medical text database to attempt to identify the driver mutation, which would be the target for personalized treatment.

### Biosimulation Approach to Personalizing Treatment of Brain Cancer

Gene Network Sciences (GNS), using its REFS™ (Reverse Engineering and Forward Simulation) technology, is collaborating of with M.D. Anderson Cancer Center (Houston, TX) to translate DNA sequence and clinical data from GBM patients into breakthrough discoveries leading to drugs and diagnostics. The results from these projects will include the identification of new combination drug targets for disease and the development of diagnostics to determine appropriate individual patient treatments. The parties plan to transform this coherent clinical 3D Data into computer models which link genetic alterations to changes in gene expression to progression-free patient survival times. This computer model, developed by using the REFS™ platform, is expected to unravel the complex genetic circuitry underlying GBM and reveal novel drug targets and biomarkers of response. These targets and biomarkers may be used to identify the optimal single or combination drug

therapy for a given patient's genetic alteration profile. The parties will utilize M.D. Anderson's clinical expertise to validate the discoveries and will work with strategic partners to make drugs and diagnostics stemming from these discoveries available to patients.

### Companion Diagnostic for Viral Gene Therapy of Brain Cancer

Tova 511 (vocimagene amiretrorepvec), an injectable, and Toca FC (flucytosine), an extended-release tablet, are formulations of a retroviral replicating vector for delivering a cytosine deaminase gene selectively to cancer cells. After Toca 511 spreads through a tumor, the cancer cells expressing the cytosine deaminase gene may convert the antibiotic flucytosine into the anticancer drug 5-FU. Tocagen, manufacturer of Toca 511 and Toca FC, has givens Siemens Healthcare Diagnostics commercialization rights to diagnostic tests for monitoring the levels of viral gene therapy for brain cancer. Tocagen is enrolling patients for its clinical trials and will partner with Siemens on assays used for the trials.

### Drug Resistance in GBM

Despite their nearly universal activation of mammalian target of rapamycin (mTOR) signaling, GBMs are strikingly resistant to mTOR-targeted therapy. Analysis of GBM cell lines, patient-derived tumor cell cultures, and clinical samples from patients in phase I clinical trials, has revealed that the promyelocytic leukemia (PML) gene mediates resistance to mTOR-targeted therapies (Iwanami et al. 2013). Direct mTOR inhibitors and EGF receptor (EGFR) inhibitors that block downstream mTOR signaling promote nuclear PML expression in GBMs. Genetic overexpression and knockdown approaches demonstrate that PML prevents mTOR and EGFR inhibitor-dependent cell death. Low doses of the PML inhibitor, arsenic trioxide, abrogate PML expression and reverse mTOR kinase inhibitor resistance in vivo, thus markedly inhibiting tumor growth and promoting tumor cell death in mice. These results identify a unique role for PML in mTOR and EGFR inhibitor resistance and provide a strong rationale for a combination therapeutic strategy to overcome it.

Intratumor heterogeneity of glioblastoma multiforme is likely the key to understanding treatment failure or drug resistance. An integrated genomic analysis of spatially distinct tumor fragments has been developed to uncover extensive intratumor heterogeneity (Sottoriva et al. 2013). Phylogeny of the fragments for each patient was reconstructed by identifying copy number alterations in EGFR and CDKN2A/B/p14ARF as early events, and aberrations in PDGFRA and PTEN as later events during cancer progression. Results of the study revealed patient-specific patterns of cancer evolution, to enable more effective personalized treatment design.

**Genetics and Genomics of Brain Cancer**

Genetic alterations in GBM have been studied extensively using molecular diagnostic technologies. Gene expression profiling reveals extensive differences in gene expression among GBMs, particularly in genes involved in angiogenesis, immune cell infiltration, and extracellular matrix remodeling. One gene, FABP7, is associated with survival and is a prognostic marker of both biologic and clinical significance. Several types of deletions of chromosome 1 have been identified but only the complete loss of the short arm of chromosome 1 combined with complete loss of the long arm of chromosome 19 signifies a good prognosis. Partial loss of the short arm of chromosome 1, on the other hand, characterized more aggressive tumors. These findings are recorded by using high-density array-comparative genomic hybridization (CGH) analysis. By using these tools, physicians can revamp and refine tumor classification to enable more individualized treatment. Expression profiling combined with mutation analysis has an important role in the development of rational therapies for GBM.

Genetic differences may also have indirect effects on drug response that are unrelated to drug metabolism or transport, such as methylation of the methylguanine methyltransferase (MGMT) gene promoter, which alters the response of GBM to treatment with carmustine. The mechanism of this effect is related to a decrease in the efficiency of repair of alkylated DNA in patients with methylated MGMT.

Activation of the transcription factor STAT3 is considered to potently promote oncogenesis in a variety of tumors including GBM leading to intense efforts to develop STAT3 inhibitors for treatment. However, the function of STAT3 in GBM pathogenesis has remained unknown. STAT3 is a key gene that turns neural stem cells into astrocytes during normal development. STAT3 has been reported to play a pro-oncogenic or tumor-suppressive role depending on the mutational profile of the tumor (de la Iglesia et al. 2008). Deficiency of the tumor suppressor PTEN triggers a cascade that inhibits STAT3 signaling in murine astrocytes and human GBM. Specifically, there is a direct link between the PTEN–Akt–FOXO axis and the leukemia inhibitory factor receptor β (LIFRβ)–STAT3 signaling pathway. Accordingly, PTEN knockdown induces efficient malignant transformation of astrocytes upon knockout of the STAT3 gene. Remarkably, in contrast to the tumor-suppressive function of STAT3 in the PTEN pathway, STAT3 forms a complex with the oncoprotein epidermal growth factor receptor type III variant (EGFRvIII) in the nucleus and thereby mediates EGFRvIII-induced glial transformation. In short, when EGFR is mutated, STAT3 is an oncogene; with a PTEN mutation, STAT3 is a tumor suppressor. These findings indicate that STAT3 plays opposing roles in glial transformation depending on the genetic background of the tumor, providing the rationale for personalized treatment of GBM. STAT3 has also been implicated in prostate and breast cancers, so these results may translate to other types of tumors as well.

Mutations of EGFR are found in over 50 % of GBMs. Concomitant activation of wild-type and/or mutant (vIII) EGFR and ablation of Ink4A/Arf and PTEN tumor suppressor gene function in the adult mouse CNS induces rapid onset of an

infiltrating, high-grade malignant glioma phenotype with prominent pathological and molecular resemblance to GBM in humans (Zhu et al. 2009). Studies of the activation of signaling events in these GBM tumor cells revealed notable differences between wild-type and vIII EGFR-expressing cells. Whereas wild-type EGF receptor signals through its canonical pathways, tumors arising from expression of mutant EGFRvIII do not use these same pathways. These findings provide critical insights into the role of mutant EGFR signaling function in GBM tumor biology and set the stage for testing of targeted therapeutic agents in suitable preclinical models.

A comprehensive analysis using next-generation sequencing technologies has led to the discovery of a variety of genes that were not known to be altered in GBMs (Parsons et al. 2008). There were recurrent mutations in the active site of isocitrate dehydrogenase 1 (IDH1) in 12 % of GBM patients; these occurred in a large fraction of young patients and in most patients with secondary GBMs and were associated with an increase in overall survival. These studies demonstrate the value of unbiased genomic analyses in the characterization of human brain cancer and identify a potentially useful genetic alteration for the classification and targeted therapy of GBMs.

Nuclear factor-kappaB (NF-κB) activation may play an important role in the pathogenesis of cancer and also in resistance to treatment. Inactivation of the p53 tumor suppressor is a key component of the multistep evolution of most cancers. Links between the NF-κB and p53 pathways are under intense investigation. Receptor interacting protein 1 (RIP1), a central component of the NF-κB signaling network, negatively regulates p53 tumor suppressor signaling (Park et al. 2009b). Loss of RIP1 from cells results in augmented induction of p53 in response to DNA damage, whereas increased RIP1 level leads to a complete shutdown of DNA damage-induced p53 induction by enhancing levels of cellular mdm2. The key signal generated by RIP1 to up-regulate mdm2 and inhibit p53 is activation of NF-κB. The clinical implication of this finding is shown in GBM, where RIP1 is commonly overexpressed, but not in grades II and III glioma. RIP1 activates NF-κB and then that increases the expression of the gene mdm2, which inhibits the p53 gene in GBM. Increased expression of RIP1 confers a worse prognosis. These results show a key interaction between the NF-κB and p53 pathways that may have implications for the targeted treatment of GBM. One of the next steps is to determine whether these patients may respond better to drugs targeting the NF-κB network.

### Glioma Actively Personalized Vaccine Consortium

A research consortium, Glioma Actively Personalized Vaccine Consortium (GAPVAC), consisting of 14 partners from 7 European countries plus the US was formed in 2013 (http://gapvac.eu/). Led by Immatics Biotechnologies, and supported by the EU with a grant through its 7th Framework Program, GAPVAC announced plans to develop a personalized brain tumor therapy. The GAPVAC project is designed to create, manufacture and develop actively personalized vaccines

(APVACs) tailored for each patient based on the individual aspects of the patient's tumor and immune system. The latest technologies, including next-generation sequencing, high-sensitivity MS and innovative immunomonitoring approaches, will be combined to generate an optimal therapy for the individual patient. At the core of the project is GAPVAC-101, a phase I clinical trial on newly diagnosed GBM patients, which started in 2014. Newly diagnosed GBM patients are repetitively immunized with an actively personalized peptide vaccine specifically prepared for each plus polyimmunomodulators concurrent to first line temozolomide maintenance therapy. An extensive biomarker program will investigate the mechanism-of-action and identify biomarker signature candidates to predict which patients are most likely to benefit from treatment with APVACs.

**Prognosis of Glioblastoma Multiforme Based on Its Genetic Landscape**

The alteration of multiple networking genes by recurrent chromosomal aberrations in gliomas deregulates critical signaling pathways through multiple, cooperative mechanisms (Bredel et al. 2009). These mutations, which are likely due to nonrandom selection of a distinct genetic landscape during gliomagenesis, are associated with patient prognosis.

A clinical study has shown that 14-3-3zeta positive expression was observed in approximately 74.5 % of patients with GBM who had lower overall survival rates and median survival time than those in the 14-3-3zeta negative group (Yang et al. 2011). 14-3-3zeta positive expression in tumor cells also was correlated with a shorter interval to tumor recurrence. Univariate and multivariate analyses showed that 14-3-3zeta positive expression was an independent prognostic factor for GBM and can be used as a biomarker.

GBMs often have both monosomy of chromosome 10 and gains of the EGFR gene locus on chromosome 7. Chromosome 10 losses that decrease tumor suppressor gene ANXA7 levels correspond to a rise in EGFR levels that increase tumor aggressiveness and decrease survival times. This provides a clinically relevant mechanism to augment EGFR signaling in glioblastomas beyond that resulting from amplification of the EGFR gene (Yadav et al. 2009). Further work is continuing to characterize the mechanism by which ANXA7 regulates EGFR.

Seven of the 31 most intriguing landscape genes are independently associated with patient survival in GBM: POLD2, CYCS, MYC, AKR1C3, YME1L1, ANXA7, and PDCD4. This seven-gene set could retrospectively classify patients into subgroups linked to survival times. Individuals who have alterations in between zero and two of the seven genes are classified as low risk, while those with five or more affected genes are considered high risk. Those in between are classified as high risk. This type of approach could have clinical applications both for improving brain tumor classification methods (currently based on histology and clinical factors such as age) and guiding treatment decisions. These findings will spur the development of new therapies based on key brain cancer pathways. Prospective clinical trials are planned for testing the clinical utility of the seven-gene set. A similar

genetic landscape approach may be applied to other aggressive types of cancer, such as ovarian and lung cancer. Eventually, networks may be created that account for both genetic and epigenetic changes in cancer cells.

### Molecular Diagnostics for Personalized Management of Brain Cancer

Several molecular biomarkers have been identified in diffuse gliomas that carry diagnostic and prognostic information. In addition, some of these and other biomarkers predict the response of these gliomas to particular chemotherapeutic approaches. Molecular diagnostics is an important contribution to personalized management of glioma patients.

**Diffusion MRI as a Biomarker** The response to treatment of brain cancer is usually assessed by measurements obtained from brain imaging several months after the start of treatment. A biomarker of tumor response would be useful for making early treatment decisions and for determining prognosis. To obtain this information, patients with GBM are examined by diffusion MRI before treatment and 3 weeks after treatment; the images are coregistered, and differences in tumor-water diffusion values are calculated as functional diffusion maps (fDM), which are correlated with the radiographic response, time-to-progression, and overall survival. Changes in fDM at 3 weeks are closely associated with the radiographic response at 10 weeks. The percentage of the tumor undergoing a significant change in the diffusion of water is different in patients with progressive disease as compared to those with stable disease. fDM provides an early biomarker for response, time-to-progression, and overall survival in patients with GBM. This method has the potential to evaluate differences in efficacy between patients, as well as to assess the heterogeneity of response within an individual tumor. This technique needs to be further evaluated to determine its usefulness in the individualization of treatment or evaluation of the response to treatment in clinical trials.

**Combined Neuroimaging and DNA Microarray Analysis** This method has been used to create a multidimensional map of gene-expression patterns in GBM that provides clinically relevant insights into tumor biology (Diehn 2008). Tumor contrast enhancement and mass effect can predict activation of specific hypoxia and proliferation gene-expression programs, respectively. Overexpression of EGFR, a receptor tyrosine kinase and potential therapeutic target, has also been directly inferred by neuroimaging and validated in an independent set of tumors by immunohistochemistry. Furthermore, imaging provides insights into the intratumoral distribution of gene-expression patterns within GBM. An “infiltrative” imaging phenotype can identify and predict patient outcome. Patients with this imaging phenotype have a greater tendency toward having multiple tumor foci and demonstrate significantly shorter survival than their counterparts. These findings provide an in vivo portrait of genome-wide gene expression in GBM and offer a potential strategy for noninvasively selecting patients who may be candidates for individualized therapies.

**Proteomics of Brain Cancer** Protein biomarkers of brain tumors have the potential for clinical usefulness to predict efficacy of anticancer agents. Surgical samples of human GBM can be analyzed with 2D GE and MS. In vitro chemosensitivities to various anticancer agents (e.g. cyclophosphamide, nimustine, cisplatin, cytosine arabinoside, mitomycin C, adriamycin, etoposide, vincristine, and paclitaxel) can be measured by flow cytometric detection of apoptosis. Proteins that significantly affect in vitro chemosensitivity to each category of anticancer agents are identified. Many of the proteins that correlate with chemoresistance are categorized into the signal transduction proteins including the G-proteins. Thus proteomic analysis using 2D GE could provide a list of proteins that may be the potential predictive biomarkers for chemosensitivity in human gliomas. They can also be direct and rational targets for anticancer therapy and be used for sensitization to the conventional chemotherapeutic regimens.

**Epigenetic Biomarkers of GBM** One of the most intrigued subtypes is the long-term survival GBM, which responds better to current therapies. An investigation based on molecular epigenetic, clinical and histopathological analyses was carried out to identify biomarkers useful for distinguishing long-term survival form from classic GBM (Martinez et al. 2007). It involved analysis of the promoter methylation status of key regulator genes implicated in tumor invasion (TIMP2, TIMP3), apoptosis and inflammation (TMS1/ASC, DAPK) as well as overall survival, therapy status and tumor pathological features. A methylation-specific PCR approach was performed to analyze the CpG island promoter methylation status of each gene. The results of this study indicate that, compared to classic GBM, long-term survival form of GBM displays distinct epigenetic characteristics, which might provide additional prognostic biomarkers for the assessment of this malignancy.

O6-methylguanine methyltransferase (MGMT) promoter methylation has been observed in a considerable proportion of all grades and subtypes of gliomas, with no significant correlation with other known genetic alterations. On extensive literature review, in both low- and high-grade gliomas, wide variability of data on the frequency of MGMT methylation and its association with other molecular alterations from various centers was noted, mostly owing to technical causes (Jha et al. 2010). This raises questions regarding the capacity of this test for use as an objective and reproducible biomarker for customized treatment in individual cases.

**Multigene Predictor of Outcome in GBM** No single biomarker is a predictor of outcome in GBM. An analysis using GBM microarray data from four independent data sets of the genes consistently associated with patient outcome revealed a consensus 38-gene survival set (Colman et al. 2010). Worse outcome was associated with increased expression of genes associated with mesenchymal differentiation and angiogenesis. Application to FFPE samples using real-time RT-PCR assays resulted in a 9-gene subset which appeared robust in these samples. This 9-gene set was then validated in an additional independent sample set. Multivariate analysis confirmed that the 9-gene set was an independent predictor of outcome after adjusting for clinical factors and methylation of the methyl-guanine methyltransferase promoter. The 9-gene profile was also positively associated with biomarkers of

glioma stem-like cells, including CD133 and nestin. Finally, a multigene predictor of outcome in GBM was identified, which is applicable to routinely processed FFPE samples. The profile has potential clinical application both for optimization of therapy in GBM and for the identification of novel therapies targeting tumors refractory to standard therapy. The assay is commercially available as DecisionDx-GBM (Castle Biosciences Inc).

**IDH1 Genotype and Survival After Surgical Resection of GBM** Survival benefit associated with surgical resection differs based on IDH1 genotype in GBM. Therapeutic benefit from maximal surgical resection, including both enhancing and non-enhancing tumor, may contribute to the better prognosis observed in the IDH1 mutant subgroup. Thus, individualized surgical strategies for malignant astrocytoma may be considered based on IDH1 status (Beiko et al. 2014).

### Personalized Chemotherapy of Brain Tumors

Although ~26 % of patients treated with temozolomide survive >2 years, it is difficult to predict who would respond to therapy. A number of tests are used to determine the responsiveness of GBM to chemotherapy.

**Gene Promoter Methylation Testing** The O6-methylguanine-DNA methyltransferase (MGMT) gene is located at chromosome 10q26 and codes for a DNA repair enzyme that counteracts the effects of alkylating chemotherapy. In GBM patients the MGMT gene is usually inactivated due to aberrant methylation of its promoter region. Assessment of the MGMT promoter methylation status is clinically relevant as a bi0marker of response to alkylating chemotherapy and prolonged survival of GBM patients. MGMT promoter methylation testing has also been used as a biomarker for patient selection in clinical trials, e.g., CENTRIC trial that was specifically focused on patients with MGMT promoter-methylated GBM. MGMT promoter methylation is a favorable prognostic biomarker independent of the type of therapy, i.e., radio- or chemotherapy (Riemenschneider et al. 2010). Several methods are being used to assess MGMT promoter methylation in clinical samples. MGMT gene test (MDxHealth Inc) is used as a Laboratory Developed Test or as Investigational Use Only tool in the assessment of patients participating in clinical trials. Future clinical trials will determine, the degree to which MGMT promoter methylation is predictive or prognostic for each subtype of glioma, and if testing should be used in practice for the management of patients with GBM.

**Molecular Determinants of Response to EGFR Inhibitors** EGFR is amplified, overexpressed, or mutated in 50 % of GBM cases, but only 10–20 % of patients have a response to EGFR kinase inhibitors. In patients with recurrent GBM, coexpression of EGFRvIII and PTEN by tumor cells is associated with responsiveness to EGFR kinase inhibitors. One inherent resistance mechanism that faces use of EGFR inhibitors in GBM is the coactivation of multiple receptor tyrosine kinases, which generates redundancy in activation of phosphoinositide-3′-kinase (PI3K) signaling.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor is frequently phosphorylated at a conserved tyrosine residue, Y240, in GBM clinical samples (Fenton et al. 2012). Phosphorylation of Y240 is associated with shortened overall survival and resistance to EGFR inhibitor therapy in GBM patients and plays an active role in mediating resistance to EGFR inhibition in vitro. Y240 phosphorylation can be mediated by both FGF receptors and SRC family kinases but does not affect the ability of PTEN to antagonize PI3K signaling. These findings show that, in addition to genetic loss and mutation of PTEN, its modulation by tyrosine phosphorylation has important implications for the development and treatment of GBM.

**Simulating Chemotherapeutic Schemes for Individualization** A novel patient individualized, spatiotemporal Monte Carlo simulation model of tumor response to chemotherapeutic schemes in vivo has been described (Stamatakos et al. 2006). Treatment of GBM by temozolomide is considered as a paradigm. The model is based on the patient's imaging, histopathologic and genetic data. A mesh is superimposed upon the anatomical region of interest and within each geometrical cell of the mesh the most prominent biological "laws" (cell cycling, apoptosis, etc.) in conjunction with pharmacokinetics and pharmacodynamics information are applied. A good qualitative agreement of the model's predictions with clinical experience supports the applicability of the approach to chemotherapy optimization.

**Personalized Therapy of GBM Based on Cancer Stem Cells (CSCs)** CSCs play an important role in determining GBM response to therapy. Hypoxia and stem cell maintenance pathways may provide therapeutic targets to sensitize CSCs to cytotoxic therapies to improve treatment of GBM patients. Although chemotherapy with temozolomide may contain tumor growth for some months, invariable GBM recurrence suggests that CSC maintaining these tumors persist. According to a study of the effect of temozolomide on CSC lines, although differentiated tumor cells constituting the bulk of all tumor cells were resistant to the cytotoxic effects of the substance, temozolomide induced a dose- and time-dependent decline of the stem cell subpopulation (Beier et al. 2008). Temozolomide concentrations that are reached in patients are only sufficient to completely eliminate CSC in vitro from MGMT-negative but not from MGMT-positive tumors. These data strongly suggest that optimized temozolomide chemotherapeutic protocols based on MGMT status of CSCs might substantially improve the elimination of GBM stem cells and consequently prolong the survival of patients.

## Supratentorial Hemispheric Diffuse Low-Grade Gliomas

Supratentorial hemispheric diffuse low-grade gliomas (LGG), i.e. World Health Organization (WHO) grade II gliomas, are a heterogeneous group of tumors. During their natural course, LGG tend to progress to a higher grade of malignancy, leading to neurological disability and ultimately to death. During their low-grade period, these tumors exhibit systematically a spontaneous and continuous radiological

growth, whatever their histological subtypes. The radiological tumor growth is easily quantified by measuring the evolution of the equivalent tumor diameter (calculated from the tumor volume), obtaining the Velocity of Diametric Expansion (VDE). The spontaneous VDE of LGG varies markedly with an average VDE of about 4 mm/year. It depends on intrinsic factors (1p19q codeletion status, P53 overexpression status) and can be modified by extrinsic factors such as pregnancy. VDE has a strong prognostic significance regarding progression free and overall survivals. Therefore, VDE should be integrated along with the other "static" parameters (multimodal imaging, histological and molecular analyses) in the initial investigations (Pallud et al. 2012). Assessment of VDE obtained before, during, and after cancer therapy helps in analyzing the effects on an individual basis as a guide to the decision in management.

**Personalized Therapy of Oligodendroglial Tumors (OTs)**

Oligodendroglial tumors (OTs) constitute one-third of gliomas and their distinction from astrocytic gliomas is important both for prognosis and therapy, but is often not adequately accurate. Because response to chemotherapy varies and the adverse effects may outweigh benefits in pathological types of tumors that do not respond to chemotherapy, there is thus an urgent need for refined diagnostic markers to improve glioma classification and predicting their chemosensitivity. LOH markers or in situ hybridization probes mapping to 1p36 have been used to identify chemosensitive OTs. It has become increasingly clear, however, that not all chemotherapy-sensitive OTs can be identified by this limited set of diagnostic tools, and that some OTs, despite their loss of 1p, are chemoresistant. Novel predictive diagnostic tools are being developed for personalizing the treatment of OTs by aiming to: (i) define a molecular profile capable of identifying all gliomas that are sensitive to procarbazine, lomustine, and vincristine (PCV): and (ii) to identify genes/signaling pathways involved in PCV chemosensitivity.

Anaplastic oligodendroglioma (AO) and anaplastic oligoastrocytoma (AOA) are treated with surgery and radiotherapy (RT) at diagnosis, but they also respond PCV, raising the possibility that early chemotherapy will improve survival. A randomized clinical trial showed that for patients with AO and AOA, PCV plus RT does not prolong survival. Longer progression-free survival after PCV plus RT is associated with significant toxicity. A significant finding of this trial was that tumors lacking 1p and 19q alleles are less aggressive or more responsive or both (Cairncross et al. 2006). The specific chromosomal change in oligodendroglial brain tumors is thus associated with a very good prognosis and may also identify patients who would benefit from chemotherapy treatment in addition to radiotherapy at diagnosis for long-term tumor control. The findings could change the future of how brain cancers are diagnosed and treatments are personalized based on genetic make-up of the tumor. Testing for chromosomal deletions should be a mandatory part now of the management of patients with these tumors.

Clinical implementation of these results is expected to greatly improve routine glioma diagnostics and will enable a patient specific therapeutic approach. In order to develop a routine-diagnostic test for chemosensitivity prediction that is widely applicable and cost-effective, an established multiplex ligation dependent probe amplification (MLPA) assay for OT diagnostics will be revamped by adding novel biomarkers that are identified by a combined array-approach. MLPA analysis will be performed on archival, paraffin embedded tissue of a set from clinically well-documented gliomas, and marker patterns will be identified that correlate with clinical outcome. Protocols will be established that are able to distinguish chemosensitive and chemoresistant tumors, and implementation of these protocols in routine diagnosis will enable tailored chemotherapy for individual glioma patients, thereby avoiding unnecessary harmful side effects and improving their quality of life.

**Personalized Therapy of Neuroblastomas**

Neuroblastoma usually arises in the tissues of the adrenal glands but is also seen in the nerve tissues of the neck, chest, abdomen and pelvis. It responds to chemotherapy with topotecan, which interacts with a critical enzyme in the body called topoisomerase. This enzyme helps DNA unwind so it can replicate, and topotecan inhibits its function, leading to cell death. However, finding the optimum dosage for treating neuroblastoma can be tricky.

A technique called pharmacokinetic-based (PK-based) dosing improves the response to treatment by monitoring and fine tuning topotecan drug levels. PK-based dosing reduces variability in the amount of topotecan in the body, leading to improvements in response and ultimately to improvement of the odds of survival. The aim is to get the right dosage of topotecan for a good antitumor effect and to minimize toxicity. In a prospective phase II trial, topotecan was administered with PK-guidance on a protracted schedule to achieve targeted systemic exposure and was found to be active against neuroblastoma (Santana et al. 2005). The aim of the initial treatment with the drug is to quickly reduce the size of the tumor that must be surgically removed. Reducing tumor size with topotecan and surgery also reduces the risk that the cancer will develop resistance to standard chemotherapy drugs that are administered afterward. The children with PK-guided drug administration did exceedingly well and tolerated the therapy with few ill effects. PK-based topotecan dosing is also being used for the brain tumor medulloblastoma and the eye cancer retinoblastoma. The scientists are now working on a method where they could tell pediatric oncologists that they could adjust the topotecan dosage according to patient characteristics to get a better antitumor effect and not even need to check blood levels. This would be a personalized approach to treatment.

Children with high-risk neuroblastoma have a poor clinical outcome. Vaccination with antigen-loaded dendritic cells (DCs) is being investigated for these children. Loading of DCs with apoptotic neuroblastoma cells or transfection with tumor mRNA represents promising strategies for development of individualized cancer vaccines/cancer gene therapy in treatment of neuroblastoma.

### Personalized Therapy of Medulloblastomas

Medulloblastoma is a malignant tumor of the cerebellum usually diagnosed in children at the median age of 5 years, but it may occur in young adults. Treatment is surgery followed by radiation therapy and chemotherapy, which have serious short-term and long-term adverse effects. Patients with recurrence after primary therapy have a particularly poor prognosis. The hedgehog pathway, an embryonic signaling cascade that regulates stem-cell and progenitor-cell differentiation, is involved in the pathogenesis as medulloblastoma arises from these cells. PTCH1 is an inhibitory cell-surface receptor that constitutively suppresses activation of the hedgehog pathway by inhibiting smoothened homologue (SMO) – a transmembrane protein that activates the downstream hedgehog signaling pathway. Hedgehog ligands bind to and inactivate PTCH1, de-repressing SMO and promoting pathway activation. Activation of hedgehog signaling, usually due to inactivating mutations of PTCH1, has been shown in ~30 % of medulloblastomas in humans.

GDC-0449, an orally bioavailable selective inhibitor of SMO, showed efficacy in phase I trials on patients with advanced basal-cell carcinoma, another type of tumor that is known to harbor PTCH1 mutations. It was used successfully in a patient with advanced medulloblastoma that had been refractory to multiple prior therapies (Rudin et al. 2009). However, this patient rapidly acquired resistance to GDC-0449. Identifying the mechanisms of acquired resistance to selective hedgehog pathway inhibitors in patients with medulloblastoma will be of particular interest in future studies. The development of a diagnostic biomarker for hedgehog pathway activation has been challenging because alteration of many pathway components may result in an activated phenotype. A gene-expression signature, which appears to correlate with hedgehog pathway activation in medulloblastoma, showed specific pathway activation in this patient's tumor. Testing this and other potential strategies for identifying biomarkers will be important components of future clinical trials of hedgehog pathway inhibitors.

### Personalized Management of Germ Cell Brain Tumors

A phase II study was carried to determine response to chemotherapy and survival after response-based radiation therapy (RT) in children with CNS germ cell tumors using serum or cerebrospinal fluid (CSF) biomarkers: human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) (Kretschmar et al. 2007). Children with germinomas and normal biomarkers received cisplatin + etoposide, alternating with vincristine + cyclophosphamide (CPM) whereas children with nongerminomatous tumors or with abnormal biomarkers received doubled doses of cisplatin and CPM. For germinoma patients in complete response (CR), RT was decreased from but dose was maintained in high-risk patients. Response (germinoma, 91 %; nongerminomatous, 55 %) and survival are encouraging after this regimen plus response-based RT.

### Personalized Management of Meningiomas

Meningiomas are the most common primary brain tumors and affect ~170,000 patients in the US. They are usually benign but can turn malignant in about 10 % of cases. Even benign tumors require surgery if they affect the surrounding brain tissue and disrupt neurological functions. Genomic analysis has shown that the entire genetic landscape of meningiomas can be explained by abnormalities in just 5 genes. Nearly half of atypical meningiomas were neurofibromin 2 (NF2)-mutant, an already known mutation responsible for genomic instability as well as association with malignancy and localizing to the cerebral and cerebellar hemispheres. The other four gene mutations now discovered are (Clark et al. 2013):

1. Mutations in TRAF7, a proapoptotic E3 ubiquitin ligase, were found in nearly one-fourth of all tumors. Meningiomas with these mutations are found in the skull base and are unlikely to become malignant.
2. Recurrent mutation in KLF4, a transcription factor known for its role in inducing pluripotency. It can induce stem cell formation, even in cells that have fully differentiated into a specific tissue type.
3. AKT1(E17K), a mutation known to activate the PI3K pathway, and is linked to malignancy.
4. SMO mutations, which activate Hedgehog signaling, were identified in ~5 % of non-NF2 mutant meningiomas. These non-NF2 meningiomas were clinically distinctive-nearly always benign, with chromosomal stability, and originating from the skull base. SMO mutations had previously been found in basal cell carcinoma and are the target of an already approved drug for that form of skin cancer. It is feasible to use targeted chemotherapy on patients with non-NF2 mutations, especially those with recurrent or invasive meningiomas and those who are surgically at high risk. Individualized chemotherapies could also spare patients irradiation treatment, a risk factor for progression of these generally benign tumors.

Collectively, these findings identify distinct meningioma subtypes, suggesting novel avenues for targeted therapeutics. Tumors mutated with each of these genes tend to be located in different areas of the brain, which can indicate how likely they are to become malignant. Knowledge of the genomic profile of the tumors and their location in the brain make it possible for the first time to develop personalized medical therapies for meningiomas, which currently are managed only surgically.

### Future Prospects of Personalized Therapy of Malignant Gliomas

There has already been considerable progress in our understanding of what drives neoplastic growth in glial tumors. Further molecular characterization of these tumors in the future will accelerate biomarker discovery and facilitate the creation of new diagnostic categories for gliomas. Only isocitrate dehydrogenase mutation status (prognostic) and O6-methlyguanine methyl transferase methylation status

and 1p/19q co-deletion (predictive) are currently routinely used for evaluation of glioma patients by clinicians in the US and UK. However, the ongoing development of targeted therapies as mono and combination treatments necessitates the discovery of optimally predictive molecular biomarkers, which will further our understanding of these tumors. Additionally, biomarker analysis will become a major factor in glioma clinical trials, with rapid identification of putative biomarkers in early stage trials with sufficient statistical power to validate predictive associations in phase III trials. Care will therefore be required to distinguish biomarkers that provide prognostic information from those that have predictive validity. This approach enable future personalized therapeutic choices with minimal toxicity and improve clinical outcomes for patients in whom the diagnosis of a malignant glioma still portends a dismal outlook (Haynes et al. 2014).

## *Personalized Management of Breast Cancer*

Personalized management of breast cancer involves improved diagnosis and selection of therapy as well as development of personalized drugs, which are targeted and specific for cancer pathways involved in breast cancer. Ninety percent of patients with early-stage breast cancer can be cured when treated only with radiation and surgery, but another 3 % also require chemotherapy to stop the cancer from spreading elsewhere. The problem is to identify these 3 %. Most patients endure chemotherapy and its devastating side effects, even though for 90 % of them the treatment is unnecessary. Breast cancer was the first cancer where a personalized approach was identified by making a distinction between estrogen receptor positive and negative cancers. Breast cancer can be typed into the following categories with distinct differences in prognosis and response to therapy:

- Estrogen receptor (ER) positive: 65–75 % of breast cancers are ER+ and are further divided into luminal A and luminal B subtypes.
- HER2 positive constitute 15–20 % of breast cancer.
- Basaloid type constitutes 15 % of cases and includes those with BRCA1 and P53 mutations.
- Triple negative for ER, progesterone receptors (PR), and HER2 receptors.

### Developing Personalized Drugs for Breast Cancer

**Developing Drugs Targeted to Pathways Involved in Breast Cancer** Up to 75 % of breast cancer patients have an abnormality in a specific cell signaling pathway, drugs that target different molecules along that pathway may be especially effective for treating the disease. Phosphatidylinositol 3 kinase (PI3K) pathway is linked to critical growth factor receptors and is involved in programmed cell death, is aberrant at multiple levels in breast cancer, including mutations in PI3K itself or

its many downstream players, such as PTEN or AKT. There is a lot of crosstalk between the PI3K pathway and other pathways, a lot of feed-forward and feedback loops. Central nodes between these intersecting circles can be effectively targeted with drugs.

Only one PI3K pathway inhibitor is in use to date but others are increasingly being developed and tested. At least 20 different companies have recognized the importance of the pathway in breast cancer and are trying to develop drugs that target it.

In the future, breast cancer tissue samples from newly diagnosed patients can be tested for their specific PI3K pathway abnormality in order to find a drug that zeroes in on what may be that particular cancer's vulnerable point. Using those drugs in combination with other treatments such as chemotherapy may significantly advance breast cancer care.

**Rational Drug Design for Breast Cancer** Capecitabine (Xeloda, F. Hoffmann-La Roche) is an example of a rationally designed cytotoxic treatment. It is designed to generate 5-FU preferentially in tumor cells by exploiting the higher activity of the activating enzyme thymidine phosphorylase in tumors compared with healthy tissues. Tumor-specific activation has the potential to enhance efficacy and minimize toxicity. Proof of this principle is provided by clinical trial results showing that capecitabine is effective and has a favorable safety profile in the treatment of metastatic breast cancer. Breast cancer treatment thus will be determined by tumor biology as well as patient characteristics. Improved molecular characterization and greater understanding of tumorigenesis will enable more individualized treatment.

**Developing Personalized Drugs for Triple-Negative Breast Cancer** Triple-negative tumors, i.e. hormone receptor- and ERBB2-negative, account for 15 % of all breast cancers and frequently harbor defects in DNA double-strand break repair through homologous recombination, such as BRCA1 dysfunction. Whereas target-specific drugs are available for treating ERBB2-overexpressing and hormone receptor-positive breast cancers, no personalized therapy exists for, triple-negative mammary carcinomas. The DNA-repair defects characteristic of BRCA1-deficient cells confer sensitivity to poly(ADP-ribose) polymerase 1 (PARP1) inhibition, which could be relevant to treatment of triple-negative tumors. AZD2281, a PARP inhibitor, was tested in a genetically engineered mouse model (GEMM) for BRCA1-associated breast cancer (Rottenberg et al. 2008). Treatment of tumor-bearing mice with AZD2281 inhibited tumor growth without signs of toxicity, resulting in strongly increased survival. Long-term treatment with AZD2281 in this model resulted in the development of drug resistance, caused by up-regulation of Abcb1a/b genes encoding P-glycoprotein efflux pumps, which could be reversed by co-administration of the P-glycoprotein inhibitor tariquidar. Combination of AZD2281 with cisplatin or carboplatin increased the recurrence-free and overall survival, suggesting that AZD2281 potentiates the effect of these DNA-damaging agents. These results demonstrate in vivo efficacy of AZD2281 against BRCA1-deficient breast cancer and illustrate how GEMMs of cancer can be used for preclinical evaluation of novel therapeutics and for testing ways to overcome therapy resistance.

**Gene Expression Plus Conventional Predictors of Breast Cancer**

In a retrospective study, researchers combined conventional predictors of breast cancer outcomes – factors such as patient age, tumor size, and so on – with information about gene expression profiles in nearly a thousand breast cancer tumor samples (Acharya et al. 2008). Their findings suggest that incorporation of gene expression signatures into clinical risk stratification can refine prognosis and potentially guide treatment of breast cancer. Identification of subgroups may not only refine predictions about patient outcomes, but also provides information about the underlying biology and the tumor microenvironment because gene expression patterns reveal different genetic pathways that are activated or silenced in different tumors. Tumors in the high-risk group with the best outcomes tended to have low expression of cancer risk genes, chromosomal instability, etc. On the other hand, tumors that have high expression of genes associated with oncogenic pathway activation, wound healing, etc., tend to be associated with poorer outcomes. Genetic signatures within high-, medium-, and low-risk groups were associated with different responses to chemotherapy treatments. Prospective studies are needed to determine the value of this approach for individualizing therapeutic strategies.

Typically, ER-positive tumors, which are more common in older women, can be treated with drugs that inhibit estrogen production. However, not all tumors that start out estrogen-receptor positive remain so. Some estrogen-receptor positive tumors respond to anti-estrogen therapy at first, but eventually become estrogen-receptor negative and resistant to these drugs. This transition is associated with patient relapse and poor overall outcomes. It is possible to classify ER-positive tumors into low-, medium-, and high-risk groups depending on the genetic signature in the tumors after patients start treatment, rather than just looking for the specific gene signature in tumors before treatment. In case of treatment with letrozole (Novartis' Femara), a drug that blocks estrogen production, clinical trials have shown that ~10 % to 15 % of estrogen-receptor positive tumors behave in a completely hormone refractory way. This approach can predict which seemingly low-risk tumors are destined to become high risk and help guide treatment accordingly. This may eventually change the way that physicians design ER receptor positive breast cancer therapies. For example, it may be possible to target aggressive, post-surgery chemotherapy to those with higher-risk tumors.

Earlier studies at NCI using mouse models and human breast cancer populations have shown that metastasis susceptibility is an inherited trait. This same combined approach facilitated the identification of a number of candidate genes that, when dysregulated, have the potential to induce prognostic gene expression profiles in human data sets. A further series of expression profiling experiments in a mouse model of metastatic breast cancer have shown that both the tumor epithelium and invading stromal tissues contribute to the development of prognostic gene signatures (Lukes et al. 2009). Furthermore, analysis of normal tissues and tumor transplants suggests that prognostic signatures result from both somatic and inherited components, with the inherited components being more consistently predictive.

EGFR and the ER are the most dangerous combination of molecules overproduced in breast cancer. When both are overfunctioning, patients are resistant to therapy and die quickly of disease progression. A study has shown how the master gene called SRC-3 (steroid receptor coactivator 3) not only enhances estrogen-dependent growth of cancer cells by activating and encouraging the transcription of a genetic message into a protein, it also sends a signal to the activating enzyme called FAK (focal adhesion kinase) found on the cell's membrane to promote cell motility or movement, which is a key element of cancer spread or metastasis (Long et al. 2010). Overexpression of SRC-3 is found in two-thirds of breast cancers. This study shows that SRC-3 can produce an alternative form of its coactivator protein – a shorter form that is missing the part of the protein (exon) that keeps it in the nucleus. With that portion gone, it leaves the nucleus and goes into the cytoplasm and travels to the membrane, where the enzyme PAK1 (p21-activated kinase 1) phosphorylates SRC-3, enabling it to function at the membrane.

### Her2 Testing in Breast Cancer as a Guide to Treatment

The information provided by a personal genetic test might be of real value in identifying the woman whose risk for breast cancer or other cancers is likely to be amplified by oral contraceptives. Depending on the mutation, oral contraceptives can increase the risk of breast cancer and may also fail to protect against ovarian cancer. Thus, a positive test for certain genetic mutations means that the strategy of using oral contraceptives to reduce the risk of ovarian cancer should be abandoned. In contrast, a woman worried about ovarian cancer who does not have one of these hereditary contraindications could then take oral contraceptives without danger of precipitating a known hereditary breast cancer.

Women with a family history of breast cancer also have the option for prophylactic breast removal, which reduces the breast cancer risk by 90 %. Chemoprevention with tamoxifen or other agents is another option. The goal is to make chemoprevention as effective as prophylactic mastectomy.

There is evidence that some of the gene mutations in breast cancer are relevant to treatment. The human epidermal growth factor receptor-2 (HER2) gene also known in avian species as c-erbB-2 (avian *er*ythro*b*lastic leukemia viral oncogene homolog 2) or in the rat as neu (*neu*roblastoma oncogene) is amplified in 20–30 % of breast cancers. HER2 gene amplification and HER2 overexpression occur early in the development of breast cancers and are found in a high proportion of ductal carcinomas in situ (DCIS), non-invasive cancers that generally do not give rise to metastases. In DCIS, HER2 overexpression is found specifically in poorly histologically differentiated disease and not in well-differentiated cancers. HER2 expression is associated with response to trastuzumab (Herceptin) and its lack with resistance to therapy. In a randomized trial, 1 year of treatment with trastuzumab after adjuvant chemotherapy significantly improved disease-free survival among women with HER2-positive breast cancer (Piccart-Gebhart et al. 2005). The randomized, controlled Mammary5 trial by the National Cancer Institute of Canada showed that

amplification of HER2 in breast-cancer cells is associated with better clinical responsiveness to anthracycline-containing chemotherapy regimen when compared with the regimen of cyclophosphamide, methotrexate, and fluorouracil (Pritchard et al. 2006). Benefit of anti-HER2 therapies demonstrated in clinical trials indicates that HER2 is, to date, one of the most promising molecules for targeted therapy. Nevertheless, since tumor cells utilizing alternative growth signaling pathways through transmembrane receptors as well as intracellular signaling transduction molecules can bypass HER2 blockade, a future ambitious aim is the successful combination of anti-HER2 strategies with drugs directed to molecules that contribute to anti-HER2 resistance (Tagliabue et al. 2010).

Various methods that have been used to analyze the HER2 status of a tumor include the following:

- Immunohistochemistry: protein expression levels
- ELISA: shedding of HER2 receptor
- FISH: HER2 gene amplification
- Quantitative PCR: HER2 gene amplification
- Quantitative RT-PCR: mRNA expression level

In practice, immunohistochemistry is the most frequently used method. However, it is recommended that all specimens with weakly positive immunohistochemistry (+2 Hercep Test result) be evaluated by FISH for HER2/neu gene amplification. The results of both assays should be considered before making a decision to recommend anti-HER2 therapy. The LightCycler™ PCR assay (Roche) has now been developed specifically to assess HER2 gene amplification. The advantages are:

- It is accurate for determining HER2 gene amplification and correlates well with FISH; 85 % sensitivity and 95 % specificity.
- It is a rapid screening method with up to 30 samples per run
- The kit uses a reference sequence on chromosome 17 so that a correct data interpretation should be possible in polysomic cases

One limitation of LightCycler PCR is that it does not give histopathological assignment. Microdissection may be required in critical cases. The combined use of laser capture microdissection, DNA microarray, and real-time quantitative PCR technologies now provides a unique opportunity to elucidate the in vivo genetic events that underlie the initiation and progression of human breast cancer. The clinical utility of the serum test as a prognostic indicator has not yet been fully established but is under investigation.

Current methods for checking HER2 are problematic because of issues with intra- and inter-laboratory reproducibility and pre-analytic variables, such as fixation time. In addition, the commonly used HER2/chromosome 17 ratio presumes that chromosome 17 polysomy is present when the centromere is amplified, even though analysis of the rest of the chromosome is not included in the assay. In one study, 97 frozen samples of invasive lobular and invasive ductal carcinoma, with known ICH and FISH results for HER2, were analyzed by aCGH to a commercially available bacterial artificial chromosome whole-genome array containing 99 probes

targeted to chromosome 17 and the HER2/TOP2 amplicon (Yeh et al. 2009). Results were 97 % concordant for HER2 status, meeting the College of American Pathologists/American Society of Clinical Oncology's validation requirements for HER2 testing. No case of complete polysomy 17 was detected even though multiple breast cancer cases showed polysomies of other chromosomes. Therefore, aCGH is an accurate and objective DNA-based alternative for clinical evaluation of HER2 gene copy number, and that polysomy 17 is a rare event in breast cancer. It is commercially available as HerScan™ (Combimatrix Molecular Diagnostics).

### HER2/neu-Derived Peptide Vaccine for Breast Cancer

HER2-positive breast cancers, which contain more receptors than is typical, are found in 20–30 % of all breast cancer patients, are treated by trastuzumab and lapatinib that latch on to these receptors and destroy them. However, some of these patients develop resistance to these therapies or develop cancer metastasis, which are hard to treat. Vaccination is an attractive alternative approach to provide HER-2/neu-specific antibodies and may in addition concomitantly stimulate HER2-reactive T-cells. A pilot clinical trial has shown that that HER2-pDNA vaccination in conjunction with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 administration is safe, well tolerated and can induce long-lasting cellular and humoral immune responses against HER2 in patients with advanced breast cancer (Norell et al. 2010).

HER2/neu, a source of immunogenic peptides, is expressed in >75 % of breast cancer patients. Clinical trials have been conducted with the HER2/neu E75 peptide vaccine in breast cancer patients. Results show that most patients with various levels of HER2/neu expression respond immunologically and seem to benefit from vaccination (Benavides et al. 2009). E75 is predicted to bind to HLA-A3, and preclinical data support this. Another trial has demonstrated that HLA-A3 patients respond similarly to E75 vaccination as HLA-A2 patients, suggesting the potential use of the E75 vaccine in up to 76 % of the population (Patil et al. 2010). Antigen Express is developing a peptide immunotherapeutic for in patients with HER-2/neu positive breast cancer, which is currently in phase II clinical trials.

### Molecular Diagnostics in Breast Cancer

Molecular diagnosis of breast cancer is discussed in detail in another report (Jain 2015). Some of the tests that relevant to prognosis and personalized management are:

**BRACAnalysis (Myriad Genetics Inc)** This test for hereditary breast and ovarian cancers incorporates thorough full-sequence analysis for gene mutation detection. Myriad and others have discovered and published information on an additional type of mutation, known as a large rearrangement that has not been detectable by

commercial DNA sequencing technologies, but only by laborious, manual research-based methods. Such rearrangements are responsible for a small percentage of changes in the two breast cancer genes. Myriad added a panel of five common rearrangements to its BRACAnalysis test, accounting for nearly half of the total occurrence of large rearrangements in the two genes. Because large rearrangements are quite rare, a woman meeting the commonly employed selection criteria for BRACAnalysis has less than 0.5 % risk of carrying one of the large rearrangement mutations. Myriad's BRACAnalysis Rearrangement Test (BART) is an automated molecular diagnostic test in the BRACAnalysis family of products, which detects rare, large rearrangements of the DNA in the BRCA1 and BRCA2 genes and is performed in women with exceptionally high risk who have tested negative for sequence mutations and the common large rearrangements already included in Myriad's test.

**Next Generation Sequencing-Based Breast Cancer Genetic Test (NewGene Inc)** Unlike Myriad's test, BRACAnalysis, which is PCR-based, NewGene's assay uses NGS technology that results in faster turnaround times and lower costs compared to other technologies. This will lead to improved access to a breast cancer genetic test with clinical use for patients. The test is based on full gene sequencing of the BRCA1 and BRCA2 genes, so it is not targeting specific mutations. NewGene uses the Roche 454 GS-FLX platform for pyrosequencing. Unlike traditional Sanger sequencing, which involves looking at individual segments of a gene one segment at a time and one patient at a time, pyrosequencing enables the investigation of genes of interest in multiple patients in the same run and with multiple gene fragments in the same run. Thus, NewGene can look at 20,000 fragments in one run in contrast to 1 fragment per run allowed by Sanger sequencing-based methods. Because each patient requires about 100 fragments to be sequenced, the increase in the number of patients that can be investigated in a single run and the improvement in throughput achieved by this technology, are significant. Test results using this technology can be achieved in 4 weeks.

**FAST (Fiber Array Scanning Technology)** This combines laser techniques with a whiskbroom bundle of fiberoptic threads enabling accurate detection of traveling cancer cells, at a much faster pace than current screening allows. The approach also employs a digital microscope to further home in on the pinpointed cancer cells. FAST works by an ethereal method called "collecting the light." The combination of the FAST cytometer and the digital microscope can spot 98 % of the traveling cancer cells in a sample. And it produces a false positive fewer than three times in a million tries – compared with a hundred false positives in a million tries for an automated digital microscope alone – the current most accurate method. FAST cytometer, has been tested on blood samples from patients. The system someday could be used alongside mammograms for better breast cancer screening.

**Real Time Qualitative PCR (Real Time-qPCR) Assays)** These have been used to risk-stratify breast cancers based on biological 'intrinsic' subtypes and proliferation (Perreard et al. 2006). Realtime-qPCR is attractive for clinical use because it is

fast, reproducible, tissue-sparing, quantitative, automatable, and can be performed from archived (formalin-fixed, paraffin-embedded tissue) samples. The benefit of using realtime-qPCR for cancer diagnostics is that new markers can be readily validated and implemented, making tests expandable and/or tailored to the individual. For instance, the proliferation metagene could be used within the context of the intrinsic subtypes or used as an ancillary test in breast cancer and other tumor types where an objective and quantitative measure of grade is important for risk stratification. As more prognostic and predictive signatures are discovered from microarray, it should be possible to build on the current biological classification and develop customized assays for each tumor subtype. This approach enables the important clinical distinction between ER-positive and ER-negative tumors and identifies additional subtypes that have prognostic value. The proliferation metagene offers an objective and quantitative measurement for grade and adds significant prognostic information to the biological subtypes. It is a robust predictor of survival across all breast cancer patients and is particularly important for prognosis in Luminal A (ER-positive) breast cancers, which have a worse outcome than expected when proliferation is high. This supports previous findings that a genomic signature of proliferation is important for predicting relapse in breast cancer, especially in ER-positive patients.

A study has compared realtime-qPCR results for the assessment of mRNA levels of ERa, PgR, and the members of the human epidermal growth factor receptor family, HER1, HER2, HER3 and HER4 (Labuhn et al. 2006). The results were obtained in two independent laboratories using two different methods, SYBR Green I and TaqMan probes, and different primers. By linear regression a good concordance was demonstrated for all six biomarkers. The quantitative mRNA expression levels of ERa, PgR and HER2 also strongly correlated with the respective quantitative protein expression levels prospectively detected by EIA in both laboratories. In addition, HER2 mRNA expression levels correlated well with gene amplification detected by FISH in the same biopsies. These results indicate that both realtime-qPCR methods were robust and sensitive tools for routine diagnostics and consistent with standard methods. The simultaneous assessment of several biomarkers is fast as well as labor effective and optimizes the clinical decision-making process in breast cancer tissue and/or core biopsies.

**Gene Expression Profiling** Gene-expression profiling with the use of DNA microarrays enables measurement of thousands of mRNA transcripts in a single experiment. These are being used to develop new prognostic and predictive tests for breast cancer, and might be used at the same time to confirm estrogen-receptor status and ERBB2 status. Gene expression data of breast cancer samples were used to assess the correlation between estrogen receptor (ER) and ERBB2 mRNA and clinical status of these genes as established by immunohistochemistry or FISH or both (Gong et al. 2007). Amounts of ESR1 and ERBB2 mRNA, as measured by the Affymetrix U133A GeneChip, reliably and reproducibly established estrogen-receptor status and ERBB2 status, respectively. The gene expression tests are 90 % accurate for both receptors, which make them comparable to, if not better than,

existing pathology tests. This is one important step towards personalized diagnosis and treatment planning based on an integrated genomic test of an individual tumor.

Resistance to treatment with endocrine therapy occurs in ~50 % of breast cancer patients. The transcription factor PBX1, a known NOTCH target gene, is required for the growth of endocrine therapy-resistant breast cancer cells. The NOTCH pathway is overactivated in resistant breast cancer cells, whereas classical ERα signaling is epigenetically disengaged. Blocking of NOTCH signaling abrogates growth of resistant breast cancer cells. A gene expression signature based on NOTCH-PBX1 activity can determine if breast cancer patients are responsive or not to endocrine therapy (Magnani et al. 2013).

Results of gene expression studies have confirmed that breast cancer is not a single disease with variable morphologic features and biomarkers but, rather, a group of molecularly distinct neoplastic disorders. This forms the basis of molecular classification of breast cancer. Profiling results also support the hypothesis that ER-negative and ER-positive breast cancers originate from distinct cell types and point to biologic processes that govern metastatic progression. Moreover, such profiling has uncovered molecular signatures that could determine response to chemotherapy and influence clinical care of patients with breast cancer (Sotiriou and Pusztai 2009).

Unbiased NGS studies have identified several recurrently mutated genes in breast cancer that represent putative novel therapeutic targets (Russnes et al. 2011). PI3KCA was found as one of the most frequently mutated genes in breast and other cancer types. Therapeutic targeting of the PI3K/AKT signaling pathway has been a major focus of several drug companies, leading to the development and clinical testing of several PI3K and AKT inhibitors. Upregulation of phosphorylated HER3 and partial recovery of phospho-AKT has been observed following XL147 treatment, leading to incomplete suppression of tumor cell growth (Chakrabarty et al. 2012). Based on follow-up experiments, these authors demonstrated that the combined inhibition of HER2 and PI3K leads to synergistic effects and more efficient eradication of the tumors. These results are an example for the complexity of signaling pathways in cancer cells complicated by multiple layers of feedback inhibition.

**TOP 20 model**, licensed by Tiziana Life Sciences from the European Institute of Oncology for commercial development, is a gene expression signature capable of predicting disease aggressiveness and prognosis in breast cancer patients. It is different from all other signatures available today because it is derived from cancer stem cells and therefore it predicts cancer behavior based on its stem cell content. The TOP 20 genes have been defined based on published expression profiles of breast stem cells. They are further selected based on their levels of expression and likelihood of translation into practice for patient stratification in breast cancer.

### Monitoring of Circulating Tumor Cells in Metastatic Breast Cancer

Because repeated tissue biopsies are invasive, costly and impractical, assessment of tumor characteristics on circulating tumor cells (CTCs) by a peripheral blood sample as a ‘liquid biopsy’ provides a better alternative. Molecular and genomic

characterization of CTCs could contribute to personalized treatment selection (Toss et al. 2014).

A clinical study has used CellSearch™ (Veridex) to prospectively assess CTC status at baseline and after one cycle of a new line of systemic therapy, as well as changes from baseline for their utility in predicting response, progression-free and overall survival in metastatic breast cancer (Wallwiener et al. 2014). Increase of CTCs was significantly associated with progressive disease. Analysis of data revealed that prognostic factors for shorter progression free survival included persistent CTCs after one cycle, >=3rd-line therapy, and triple-negative receptor status. Presence of bone-and-visceral/local metastases in addition to these was associated with shorter overall survival. Thus, serial monitoring of CTC number is useful for determining prognosis and tailoring systemic treatment of metastatic breast cancer.

### Pharmacogenetics of Breast Cancer

Polymorphisms in tamoxifen metabolizing genes affect the plasma concentration of tamoxifen metabolites. CYP450 2D6 and CYP3A5 genotype were determined from paraffin-embedded tumor samples and buccal cells (living patients) in tamoxifen-treated women enrolled onto a North Central Cancer Treatment Group adjuvant breast cancer trial (Goetz et al. 2005). In tamoxifen-treated patients, women with the CYP2D6 *4/*4 genotype tend to have a higher risk of disease relapse and a lower incidence of hot flashes.

### Proteomics-Based Personalized Management of Breast Cancer

Nipple aspirate protein samples were taken from invasive ductal breast carcinoma and also had an apparently normal contralateral breast. These can be examined by 2D GE and mass spectrometry as well as highly sensitive staining techniques that can detect proteins in the picogram range. Among the differential expression patterns of ductal fluid proteins, some evidence of known and possibly new biomarkers and drug targets for breast cancer has been observed. The patient-to-patient variability of these differences may reflect variables in the disease structure and may prove to be of clinical diagnostic and therapeutic significance to individual patients. For example, the presence or absence of known biomarkers detected in the differences in the fluids can be used to determine the aggressiveness of the cancer (e.g. the presence or level of Cyclin E) or signal the appearance of a cancer-related genetic instability or hereditary component (e.g. the absence or level of BRCA1). However, this approach requires clinical trials for comparison with the gold standards such as mammograms, ultrasound, biopsy, nipple lavage and aspirate cytology, and serum biomarkers. The presence of known drug targets detected in the differences in the fluids may also be used in the future to indicate what drugs to use.

Despite recent advances in breast cancer therapy, women with similar types of breast cancers may respond very differently to standard treatments. The emerging field of clinical proteomics has the potential to revolutionize breast cancer therapy. The ultimate goal of clinical proteomics is to characterize information flow through protein cascades for individual patients. After the protein networks have been elucidated, drug therapies may be specially designed for each patient. Proteomic technologies of laser-capture microdissection (LCM) and reverse-phase protein arrays (RPPAs) enable scientists to analyze relative abundances of key cellular signaling proteins from pure cell populations. Cell survival and apoptotic protein pathways are currently being monitored with LCM and RPPAs at the NIH in phase II clinical trials of metastatic breast and ovarian cancers. Ultimately, proteomics will become an integral component of tracking and managing personalized breast cancer therapy.

### Predicting Response to Chemotherapy in Breast Cancer

Breast cancer patients have benefited from the use of targeted therapies directed at specific molecular alterations. Some of the methods used to identify various pathways or overexpression of some genes for predicting response to therapy are:

**Predicting Response to Trastuzumab Treatment** Trastuzumab (TCH) is active against the overexpressed HER2 oncogene in breast cancer, and several prospective, randomized trials have shown that adjuvant TCH substantially reduces rates of recurrence and death in patients with early-stage disease. Combined therapy of TCH with anthracycline (AC-T)-based regimens has been associated with cardiac toxicity. A randomized trial showed that addition of 1 year of adjuvant TCH significantly improved disease-free and overall survival among women with HER2-positive breast cancer (Slamon et al. 2011). The risk-benefit ratio favored the nonanthracycline TCH regimen over AC-T plus TCG, given its similar efficacy, fewer acute toxic effects, and lower risks of cardiotoxicity and leukemia.

A DNA probe for the HER2 gene is used to predict whether a breast cancer patient is a candidate for TCH treatment. Current medical practice requires that all patients who are considered for TCH treatment be tested for HER2 amplification or overexpression. SPOT-Light® HER2 CISH Kit (Life Technologies), which is approved by the FDA, is based on chromogenic in situ hybridization (CISH), where results are visualized under a standard bright-field microscope, as opposed to FISH tests, in which the results must be visualized using a fluorescent microscope. This specialized microscope frequently requires that the analysis is done at a reference lab. In addition, HER2 CISH test results are quantifiable; removing the subjectivity inherent in tests based on immunohistochemistry.

**Genomic Predictor of Response to Taxane-Anthracycline Chemotherapy** A prospective multicenter study was conducted to test genomic predictors for chemotherapy containing sequential taxane and anthracycline-based regimens in patients with newly diagnosed ERBB2 (HER2 or HER2/neu)-negative breast cancer (Hatzis

et al. 2011). Breast cancer treatment sensitivity was predicted using combination of signatures for (1) sensitivity to endocrine therapy, (2) chemoresistance, and (3) chemosensitivity, with independent validation and comparison with other reported genomic predictors of chemotherapy response. A genomic predictor combining ER status, predicted chemoresistance, chemosensitivity, and endocrine sensitivity and identified patients with high probability of survival following taxane and anthracycline chemotherapy.

**Use of PET to Determine Response to Chemotherapy** In patients with metastatic breast cancer, sequential 18F-FDG PET enables prediction of response to treatment after the first cycle of chemotherapy. The use of 18F-FDG PET as a surrogate endpoint for monitoring therapy response offers improved patient care by individualizing treatment and avoiding ineffective chemotherapy.

**Prediction of Response to Paclitaxel** Breast cancers show variable sensitivity to paclitaxel. Tubulin polymerization assay has been used to show that low tau expression renders microtubules more vulnerable to paclitaxel and makes breast cancer cells hypersensitive to this drug. Low tau expression, therefore, may be used as a biomarker to select patients for paclitaxel therapy. Inhibition of tau function by RNAi might be exploited as a therapeutic strategy to increase sensitivity to paclitaxel.

**Predicting the Response to Anti-estrogen Drugs** According to the NCI, about two-thirds of women with breast cancer have estrogen receptor (ER)-positive breast cancer, in which tumor growth is regulated by the natural female hormone estrogen. Estrogen is known to promote the growth of most types of breast cancer. However, another gene, the retinoblastoma tumor suppressor (RB) gene, is functionally inactivated in the majority of human cancers and is aberrant in one-third of all breast cancers. RB regulates G1/S-phase cell-cycle progression and is a critical mediator of antiproliferative signaling. RB deficiency compromises the short-term cell-cycle inhibition following cisplatin, ionizing radiation, and anti-estrogen therapy of breast cancer with drugs such as tamoxifen (Bosco et al. 2007). Specific analyses of an RB gene expression signature in human patients indicate that deregulation of this pathway is associated with early recurrence following tamoxifen monotherapy. Thus, because the RB pathway is a critical determinant of tumorigenic proliferation and differential therapeutic response, it may represent a critical basis for directing therapy in the treatment of breast cancer. The RB tumor suppressor can be used as a biomarker for how tumors will respond to anti-estrogen therapy and could become the basis for deciding how patients with ER-positive breast cancer are treated clinically. This is a way to predict when anti-estrogen drug therapies are inappropriate for patients with hormone-dependent breast cancer so that physicians can immediately begin treating the patient with alternative drugs that are more likely to succeed. However, comprehensive clinical research is needed before this new method for predicting the success of anti-estrogen drugs is applied in daily patient care.

**Role of p63/p73 Pathway in Chemosensitivity to Cisplatin** Breast cancers lacking estrogen and progesterone receptor expression and Her2 amplification exhibit

distinct gene expression profiles and clinical features, and they comprise the majority of BRCA1-associated tumors. Global gene expression profiling has uncovered previously unrecognized subsets of human breast cancer, including the "triple-negative" or "basal-like" subset characterized by a lack of ER and progesterone receptor (PR) expression, the absence of HER2 amplification, and the expression of basal epithelial markers. Triple-negative breast cancers are the most common subtype arising in patients harboring germline mutations in the breast cancer predisposition gene breast cancer 1, early onset (BRCA1). Both BRCA1-associated and the more common sporadic triple-negative tumors share similar gene expression profiles and both are refractory to commonly used chemotherapeutic agents and as a result are associated with a relatively poor prognosis. The p53 family member p63 controls a pathway for p73-dependent cisplatin sensitivity specific to these "triple-negative" tumors. A study shows that p63 is a survival factor in a subset of breast cancers and provide a novel mechanism for cisplatin sensitivity in these triple-negative cancers, and suggest that such cancers may share the cisplatin sensitivity of BRCA1-associated tumors (Leong et al. 2007).

**Targeted Therapy of Breast Cancer with AGTR1 Antagonists** To identify additional opportunities for targeted therapy, a study searched for genes with marked overexpression in subsets of tumors across a panel of breast cancer profiling studies comprising 3,200 microarray experiments (Rhodes et al. 2009). In addition to prioritizing ERBB2, the researchers found AGTR1, the angiotensin II receptor type I, to be markedly overexpressed in 10–20 % of breast cancer cases across multiple independent patient cohorts. Validation experiments confirmed that AGTR1 is highly overexpressed, in several cases more than 100-fold. AGTR1 overexpression was restricted to estrogen receptor-positive tumors and was mutually exclusive with ERBB2 overexpression across all samples. Ectopic overexpression of AGTR1 in primary mammary epithelial cells, combined with angiotensin II stimulation, led to a highly invasive phenotype that was attenuated by the AGTR1 antagonist losartan. Similarly, losartan reduced tumor growth by 30 % in AGTR1-positive breast cancer xenografts. Taken together, these observations indicate that marked AGTR1 overexpression defines a subpopulation of ER-positive, ERBB2-negative breast cancer that may benefit from targeted therapy with AGTR1 antagonists, such as losartan.

**NQO1 Enzyme-Based Test for Response to Anthracycline Chemotherapy** NQO1 enzyme was shown in a Helsinki University study to protect cells against oxidative stress, and patients having one variant of the protein, NQO1*2, had worse survival chances when they were treated with an anthracycline-based chemotherapy compared with an alternative therapy. Women in the study who possessed a double copy of the NQO1*2 variant in their genome had only a 17 % survival rate while those with only a single copy or without the variant had a survival rate of 75 %. DNA Repair Company has licensed the exclusive North American rights to a test from Helsinki University and plans to use a variant of the NQO1 enzyme to create personalized medicine tests.

**Preoperative Endocrine Prognostic Index (PEPI Score)** is a predictive measurement that could help many women diagnosed with early-stage breast cancer avoid

chemotherapy after surgery by identifying them as having little risk of a relapse (Ellis et al. 2008). About 83 % of patients are cured of breast cancer, but 17 % are resistant to current treatments. The PEPI score is derived from tumor characteristics that are present after women with stage 2 and 3 breast cancer undergo 4 months of anti-estrogen therapy before having breast surgery. The PEPI score considers the size of the breast tumor, whether cancer is present in nearby lymph nodes, how fast tumor cells are multiplying, and whether tumors lose their estrogen receptors. Women with a PEPI score of 0 have almost nil risk of cancer recurrence during the 5-year follow-up. They could safely avoid taking chemotherapeutic agents after surgery. Women with PEPI scores of 4 or above are at very high risk of having their cancer recur and should be given all appropriate post-surgical treatments.

**Decreased Breast Density as a Biomarker of Response to Tamoxifen** Increased breast density on mammography is the leading risk factor for breast cancer, apart from age. The International Breast Intervention Study I (IBIS-I), a trial of tamoxifen for ER-positive breast cancer prevention conducted at the Cancer Research UK Centre for Epidemiology, Mathematics and Statistics in London has shown that a reduction in breast density of at least 10 % may predict who benefits from the breast cancer preventive effects of tamoxifen. Those with reduced breast density after 12 to 18 months of treatment had a 52 % reduced risk of breast cancer. By contrast, those women who did not have a decrease in breast density had only an 8 % risk reduction.

**Measurement of Estrogen Receptor mRNA to Predict Response to Tamoxifen** Quantification of mRNA has historically been done by RT-PCR. A robust method of detection of mRNA utilizing ISH has been described that is linear and shows high specificity with low background. AQUA method of quantitative immunofluorescence (QIF) has been tested for measuring mRNA in situ using ESR1 alpha gene in breast cancer to determine its predictive value compared to ER protein (Bordeaux et al. 2012). mRNA for ER (ESR1) and Ubiquitin C (UbC) were visualized using RNAscope probes and levels were quantified by quantitative ISH (qISH) on two Yale breast cancer cohorts on tissue microarrays. ESR1 levels were compared to ER protein levels measured by QIF using the SP1 antibody. Results showed that ESR1 mRNA is reproducibly and specifically measurable by qISH on tissue collected from 1993 or later. ESR1 levels were correlated to ER protein levels in a non-linear manner on two Yale cohorts. High levels of ESR1 were found to be predictive of response to tamoxifen in a manner different from value of ER.

**Prediction of Response to Chemotherapy by Intrinsic Subtypes** A 50-gene subtype predictor was developed using microarray and quantitative RT-PCR to improve on current standards for breast cancer prognosis and prediction of chemotherapy (Parker et al. 2009). It incorporates the gene expression-based intrinsic subtypes luminal A, luminal B, HER2-enriched, which are generally considered types with a poor prognosis. Breast cancer experts also typically identify a fifth breast cancer type known as normal-like. The 50-gene set also recognizes the normal-like type, but instead of being a fifth type of breast cancer, the normal-like classification is an

indicator that a sample contains insufficient tumor cells to make a molecular diagnosis and that a new sample needs to be taken.

The genetic test was highly sensitive and very predictive for chemotherapy response. The test was more predictive than typically used clinical molecular markers such as estrogen receptor status, progesterone receptor status or HER2 gene expression status. Luminal A was found to be not sensitive to the chemotherapy, suggesting that patients with this good-prognosis type can forgo chemotherapy in favor of hormone-based therapy. Among the poor-prognosis tumor types, basal-like breast cancer was the most sensitive to the chemotherapy and luminal B the least.

Diagnosis by intrinsic subtype adds significant prognostic and predictive information to standard parameters for patients with breast cancer. The prognostic properties of the continuous risk score will be of value for the personalized management of node-negative breast cancers. The subtypes and risk score can also be used to assess the likelihood of efficacy from neoadjuvant chemotherapy. This new genomic test is broadly applicable for all women diagnosed with breast cancer. Their 50-gene set can be assayed in preserved tumor samples left over from standard diagnostic procedures, so that tumor samples from breast cancer cases going back a decade or more can be studied. Since the patients in these cases have already been treated, the researchers can quickly discover how well various therapies worked for each breast cancer type. The genomic test technology will be distributed through University Genomics, a company co-owned by Washington University, the University of Utah and the University of North Carolina.

**Subtyping Breast Cancer to Predict Response to Chemotherapy** Breast cancers are comprised of molecularly distinct subtypes that may respond differently to pathway-targeted therapies now under development. Collections of breast cancer cell lines mirror many of the molecular subtypes and pathways found in tumors, suggesting that treatment of cell lines with candidate therapeutic compounds can guide identification of associations between molecular subtypes, pathways, and drug response (Heiser et al. 2012). In a test of 77 therapeutic compounds, the authors found that nearly all drugs showed differential responses across these cell lines, and approximately one third showed subtype-, pathway-, and/or genomic aberration-specific responses. These observations suggest mechanisms of response and resistance and may facilitate efforts to develop molecular assays that predict clinical response.

### Prediction of Resistance to Chemotherapy in Breast Cancer

It is well known that some breast tumors acquire altered genes or chromosomes during the course of treatment that make them resistant to many cancer drugs. With a few exceptions, e.g. ER-sensitive HER2-positive cancer, no tests are done before treatment begins to predict who is going to be resistant or sensitive to chemotherapy. Most breast cancer patients are initially given the same drugs. In search of genetic alterations that might explain disease recurrence despite treatment with adjuvant

chemotherapy in some breast cancer patients, scientists at Dana-Farber Cancer Institute (Boston, MA) scanned the genome of stored breast cancer samples from patients who had been treated according to modern guidelines, including the use of anthracyclines. Use of integrated genomics enabled identification of a small number of overexpressed and amplified genes from chromosome 8q22 that are associated with early disease recurrence despite anthracycline-based adjuvant chemotherapy (Li et al. 2010). The association was confirmed in an analysis of multiple independent cohorts. SiRNA-mediated knockdown of either of two of these genes, the anti-apoptotic gene YWHAZ and a lysosomal gene LAPTM4B, sensitized tumor cells to anthracyclines, and overexpression of either of the genes induced anthracycline resistance. Overexpression of LAPTM4B resulted in sequestration of the anthracycline doxorubicin, delaying its appearance in the nucleus. Overexpression of these two genes was associated with poor tumor response to anthracycline treatment in a neoadjuvant chemotherapy trial in women with primary breast cancer. These results suggest that 8q22 amplification and overexpression of LAPTM4B and YWHAZ contribute to de novo chemoresistance to anthracyclines and allow metastatic recurrence. Overexpression of these two genes may predict anthracycline resistance and influence selection of chemotherapy. These findings could lead to a genetic test of breast cancers to help physicians choose the best initial treatment for an individual patient that is less likely to lead to development of resistance. Such a test should not be difficult to develop and could be available for clinical testing in the near future. Testing prior to start of chemotherapy would help to personalize the treatment and reduce the possibility of development of resistance.

The 78-kDa glucose-regulated protein (GRP78), widely used as an indicator of the unfolded protein response (UPR), is induced in the tumor microenvironment. In vitro studies suggest that GRP78 confers chemoresistance to topoisomerase inhibitors, such as Adriamycin (doxorubicin) used for the treatment of breast cancer. In a retrospective study of breast cancer patients who were treated with Adriamycin, archival tumor specimens were analyzed and the relationship of GRP78 expression level to “time to recurrence” (TTR), used as a surrogate biomarker for drug resistance, was examined (Lee et al. 2006). The data show that 67 % of the study subjects expressed high level of GRP78 in their tumors before the initiation of chemotherapy and suggest an association between GRP78 positivity and shorter time to recurrence. The use of GRP78 as a predictor for chemoresponsiveness and the potential interaction of GRP78 and/or the UPR pathways with taxanes warrant larger studies.

An experimentally derived IFN-related DNA damage resistance signature (IRDS) is associated with resistance to chemotherapy and/or radiation across different cancer cell lines (Weichselbaum et al. 2008). The IRDS genes STAT1, ISG15, and IFIT1 all mediate experimental resistance. Clinical analyses reveal that $IRDS^{+}$ and $IRDS^{-}$ states exist among common human cancers. In breast cancer, a seven gene-pair classifier predicts for efficacy of adjuvant chemotherapy and for local-regional control after radiation. By providing information on treatment sensitivity or resistance, the IRDS improves outcome prediction when combined with standard markers, risk groups, or other genomic classifiers.

### Prediction of Adverse Reaction to Radiotherapy in Breast Cancer

Radiotherapy is a very important treatment for breast cancer but a small number of patients can develop severe side effects. Although fibrosis, telangiectasia and atrophy, all contribute to late radiation injury, they have distinct underlying genetic and radiobiological causes. Fibrosis risk is associated with an inflammatory response, whereas telangiectasia is associated with vascular endothelial cell damage. There is no test at present for an abnormal reaction to radiotherapy. A combined analysis of two UK breast cancer patient studies shows that 8 % of patients are homozygous for the TGFβ1 (C-509T) variant allele and have a 15-fold increased risk of fibrosis following radiotherapy (Giotopoulos et al. 2007). Atrophy is associated with an acute response, but the genetic predisposing factors that determine the risk of an acute response or atrophy have yet to be identified. Identification of the two genes associated with adverse reaction to cancer treatment means that patients who might react badly to radiotherapy could be warned in advance or alternative treatments can be sought. Further research is needed as the genes responsible for redness and peeling of the skin during treatment have not been found.

### Prediction of Recurrence in Breast Cancer for Personalizing Therapy

To tailor local treatment in breast cancer patients there is a need for predicting ipsilateral recurrences after breast-conserving therapy. After adequate treatment (excision with free margins and radiotherapy), young age and incompletely excised extensive intraductal component are predictors for local recurrence. Gene expression profiling (wound-response signature, 70-gene prognosis profile (Agendia's MammaPrint test) and hypoxia-induced profile) can identify subgroups of patients at increased risk of developing a local recurrence after breast-conserving therapy.

Lymph node status at the time of diagnosis of breast cancer is considered to be the most important measure for future recurrence and overall survival. It is an imperfect method because a third of patients with no detectable lymph-node involvement will develop recurrent disease within 10 years. DNA microarray analysis of primary breast tumors and classification to identify a gene expression signature is strongly predictive of a short interval to distant metastases in patients without tumor cells in local lymph nodes at time of diagnosis. The poor prognosis signature consists of genes regulating cell cycle, invasion, metastasis and angiogenesis. This gene expression profile will be superior to currently used clinical parameters in predicting disease outcome and selection of patients who would benefit from adjuvant therapy. The ability to accurately predict long-term recurrence with microarrays, however, might prove very important if subsets of patients who will not relapse can be spared the toxicity of adjuvant chemotherapy.

**Oncotype DX™ Breast Cancer Assay** (Genomic Health Inc), a clinically validated multigene RT-PCR test, is available for use in clinical practice to quantify the likelihood of breast cancer recurrence for an individual patient. The assay, performed using formalin-fixed, paraffin-embedded tissue, analyzes the expression of

a panel of 21 genes using RT-PCR. The likelihood of distant recurrence in patients with ER-positive breast cancer without involvement of lymph nodes is poorly defined by clinical and histopathological measures. Analysis of RT-PCR profiles obtained from tumor blocks show that recurrence score is predictive of overall survival in individual tamoxifen-treated patients with node-negative, estrogen-receptor-positive breast cancer.

**Prosigna Breast Cancer Prognostic Gene Signature Assay** (NanoString) provides digital readout of expression of 50 genes implicated in the growth and spread of cancer. The score is used to estimate the chance that cancer may recur after hormone therapy.

***The MammaPrint Test*** **(Agendia)** This FDA-approved 70-gene microarray assay is used to provide important prognostic information for individuals with primary invasive breast cancer with lymph node negative disease of either positive or negative estrogen receptor status. The microarray assay looks at what specific genes are expressed in a patient's tumor. When compared to clinical factors currently used by physicians in the prognosis of breast cancer such as age, tumor size, lymph-node status, tumor grade and estrogen receptor status, the MammaPrint test has shown to provide the best single prognostic information concerning the development of distant metastases. Large-scale prospective clinical trials of the breast cancer prognosis test have been carried out. MammaPrint test outperformed the clinicopathologic risk assessment in predicting all endpoints and adds independent prognostic information to clinicopathologic risk assessment for patients with early breast cancer as well. To facilitate its use in a diagnostic setting, the 70-gene prognosis profile was translated into a customized MammaPrint containing a reduced set of 1,900 probes suitable for high throughput processing. RNA of 162 patient samples from two previous studies was subjected to hybridization to this custom array to validate the prognostic value. Classification results obtained from the original analysis, when compared to those generated using the algorithms based on the custom mini-array, show a high correlation of prognosis prediction. Therefore, the array is an excellent tool for predicting outcome of disease in breast cancer.

***TargetPrint*®** **(Agendia)** This FDA approved test enables quantitative determination of gene expression levels of the estrogen receptor, progesterone receptor and HER2 in breast cancer biopsies. This is of paramount importance in planning treatment of breast cancer patients after surgery and assists physicians and patients in making informed treatment decisions. TargetPrint runs on Agendia's High Density Chip.

**TOP2A FISH pharmDx Test** (Dako) uses FISH to detect or to confirm abnormalities in the topoisomerase 2 alpha gene, which is involved in DNA replication. Changes in this gene in breast cancer cells can be used to predict likelihood of tumor recurrence or long-term survival of a patient. The FDA approved this test in 2008 with the remark that this is the first test to be approved that targets the TOP2A gene in cancer patients. The FDA has deemed the test suitable for premenopausal patients or those who have other indicators of higher chances of tumor recurrence, such as tumor size or lymph node involvement, or decreased survival. The test was studied

in Danish patients who were treated with chemotherapy after removal of breast cancer tumors. That study used data from tumor samples and clinical data from 767 patients with high-risk tumors, and it confirmed that the test was useful in estimating recurrence and survival in women who had received chemotherapy. Dako received the CE mark for the test in 2007 and has since launched the assay in Europe and in the US.

### Prognostic Tests for Breast Cancer

A study has demonstrated that a history of hypertension, ER/PR status, HER2 status, metastasis-free interval, metastatic location (including brain, bone and liver), and BMI at diagnosis with metastatic breast cancer were the most relevant prognostic factors for survival after diagnosis of metastatic disease (Jung et al. 2012). Findings of this study may form a foundation for the growing body of knowledge explaining the outcome differences in treatment of patients with metastatic breast cancer, potentially helping to create tailored counseling and personalized treatment approaches for this vulnerable group.

Prognostic testing of all patients prior to treatment aligns with standard medical practice to distinguish patients by hormone status. This information also enables pharmaceutical companies to clearly define patient stratification for improving clinical trial timelines and outcomes.

**Exagen's Breast Cancer Prognostic Marker Assays** These are the first and only tests to enable specific testing for hormone receptor (including estrogen receptor and progesterone receptor) positive and for hormone receptor negative patients using an improved FISH assay. These prognostic tests separate patients with good prognosis from those with poor prognosis by testing each patient's tumor tissue to detect changes in DNA (e.g. gene copy number) in order to directly reflect changes in the tumor. Exagen's prognostic tests are uniquely developed as separate sets of DNA markers to identify prognosis in hormone positive and hormone negative patients, respectively. Both marker sets represent the first prognostic tests that can be used by any FISH-testing laboratory, enabling fit of this testing approach with standard hormone testing prior to treatment. Exagen's small, prognostic marker sets combine to form a testing panel that differs from other existing sets of 20- to 70-gene markers by enabling:

- Use of improved FISH technology with a small (3–5) number of probes to fit with current laboratory testing practices and equipment;
- Testing of all breast cancer patients to provide additional prognostic information based on hormone receptor status (including estrogen receptor and progesterone receptor) prior to treatment; and
- Detection and visualization of tumor-based cellular changes to define only those DNA changes that are specific to tumor tissue.

**Prognostic Gene Biomarkers of Breast Cancer** Three genes, homeobox 13 (HOXB13), interleukin-17B receptor (IL17BR) and CHDH, and the HOXB13:IL17BR ratio index in particular, strongly predict clinical outcome in breast cancer patients receiving tamoxifen monotherapy. HOXB13:IL17BR index is a strong independent prognostic factor for ER+ node-negative patients irrespective of tamoxifen therapy. These two biomarkers serve are the foundation of the AviaraDx Breast Cancer Profiling Technology.

Activity of a gene, Dachshund (DACH1), which normally regulates eye development and development of other tissues, commandeers cancer-causing genes and returns them to normal. DACH1 inhibits the expression of the cyclin D1 gene, an oncogene that is overexpressed in about half of all breast cancers. DACH1 correlates with tumor size, stage and metastasis, and its expression is markedly reduced in metastatic breast cancer cells, but increased nuclear DACH1 expression predicts improved patient survival. DACH1 gene reverses the cancerous phenotype, thus turning the cell back to a premalignant state, and it could be used as a prognostic biomarker for breast cancer. Other genes that determine cell fate- are being examined in an attempt to identify new therapeutics for breast cancer and metastasis.

The gene CEACAM6 (carcinoembryonic antigen-related cell adhesion molecule 6) is involved in the spread of breast cancer that has developed resistance to long-term estrogen deprivation. It may prove to be a useful biomarker for predicting, which patients have the greatest risk of breast cancer recurrence, so their physicians can offer the most appropriate treatment plan. The research focused on breast cancer cells that had grown resistant to aromatase inhibitors (AIs), anti-hormone drugs to shut down the enzyme aromatase, which lets the body produce estrogen outside the ovaries. These drugs represent one of the most effective forms of hormone therapy for postmenopausal women whose breast cancer tests positive for ERs, which means that estrogen in the body fuels the growth of cancer cells. Unfortunately, one of the drawbacks to extended use of an AI may be that some of the cancer cells develop resistance to the drug and are able to grow and spread independent of estrogen. Several AI-resistant breast cancer cell lines have been developed in the laboratory and found to be very invasive compared to AI-sensitive breast cancer cells. Analyses of gene activity in these AI-resistant cells shows that they express high levels of genes associated with invasiveness and metastasis. However, this aggressive behavior could be reversed by using siRNAs to knock out the CEACAM6 gene. This gene might not only be an important biomarker for metastasis but a possible target for novel therapies for patients with metastatic breast cancer.

ER-negative basal breast cancer is a heterogeneous disease with at least four main subtypes. Heterogeneity in the clinical outcome of ER- breast cancer is related to the variability in expression levels of complement and immune response pathway genes independently of lymphocytic infiltration (Teschendorff et al. 2007).

**Multi-gene Expression Prognostic Constellation (Celera)** The prognostic constellation provides information that is distinct from that predicted by routine clinical assessment tools, such as tumor grade, and can quantify risk for metastasis for variable time periods rather than only categorically for 5 or 10 years. A previously

developed 14-gene metastasis score that predicts distant metastasis in breast cancer research subjects without systemic treatment has now been applied to Tamoxifen-treated research subjects. Many of the genes in this constellation are involved in the p53 and TNF signaling pathways and are implicated in cancer proliferation. The absence of the estrogen receptor gene in the constellation increases the confidence that this information complements routinely assayed estrogen receptor levels determined by immunohistochemistry. The test can be used as a predictor of distant metastasis in Tamoxifen®-treated breast cancer patients. A key finding is the calculation of a Metastasis Score for breast cancer that predicts a 3.5-fold difference in risk between the 20 % of women at highest risk and the 20 % of women at lowest risk.

**Insight™Dx Mammostrat™ (Clarient Inc/GE Healthcare)** This has been clinically validated as a prognostic test for women with early-stage, hormone-receptor-positive breast cancer. It combines three traditional pathology staging risk factors – tumor size, tumor grade, and lymph node status – with seven key molecular biomarkers, which include ER, PR, HER2, EGFR, BCL2, p53, and MYC. The information is then combined with a proprietary algorithm to produce a risk score that assists pathologists and oncologists in clinical decision-making. Clarient conducted an independent study using a set of breast cancer patients from the Royal Perth Hospital in Western Australia to clinically validate the Clarient Insight Mammostrat™. The algorithm demonstrated an accurate, actionable risk recurrence score. In the study, high- and low-risk patients were identified using the Clarient Insight Mammostrat™. The low-risk group had only a 3 % recurrence rate 10 years after surgery. This is equivalent to a negative predictive value of about 97 %, and the corresponding positive predictive value was 39 %. Further details can be seen on the following web site: www.clarientinc.com/mammostrat-overview.aspx.

**TaqMan Non-coding RNA Assays (Life Technologies)** These assays have helped to uncover regulatory roles of non-coding RNAs in breast cancer. Long intervening non-coding RNAs (lincRNAs) in the HOX loci become systematically dysregulated during breast cancer progression. The lincRNA termed HOTAIR is increased in expression in primary breast tumors and metastases, and HOTAIR expression level in primary tumors is a powerful predictor of eventual metastasis and death (Gupta et al. 2010). Enforced expression of HOTAIR in epithelial cancer cells induced genome-wide re-targeting of Polycomb repressive complex 2 (PRC2) to an occupancy pattern more resembling embryonic fibroblasts, leading to altered histone H3 lysine 27 methylation, gene expression, and increased cancer invasiveness and metastasis in a manner dependent on PRC2. Conversely, loss of HOTAIR can inhibit cancer invasiveness, particularly in cells that possess excessive PRC2 activity. TaqMan non-coding RNA assays can accurately measure expression levels of this molecular biomarker in different breast cancer samples.

**MetaStat™ Breast Cancer Test** Scientists at MetaStat Inc have discovered the micro-anatomical site in breast cancer by direct visual observation, the MetaSite, the window in the blood vessels through which the metastatic cells squeeze through

to enter the blood stream to begin their deadly journey. The number of these "windows" correlated to the probability of distant site metastases. MetaStat™ Breast Cancer Test uses conventional staining techniques to count these windows, and the count correlates to the risk of metastasis. In clinical trials, the high-risk cohort proved to be 22 times as likely to experience metastasis as the low. The test is inexpensive and fast because archived human tissue samples are used accompanied by their corresponding medical records. The predictions are compared to known outcomes in the corresponding medical records.

### Racial Factors in the Management of Breast Cancer

Gene expression analysis has identified several breast cancer subtypes, including basal-like, human epidermal growth factor receptor-2 positive/estrogen receptor negative (HER2+/ER–), luminal A, and luminal B. The basal-like breast cancer subtype was more prevalent among premenopausal African American women (39 %) compared with postmenopausal African American women (14 %) and non–African American women (16 %) of any age (Carey et al. 2006). Although breast cancer is less common in blacks than whites, when black women do develop the disease, they are more likely to die from it, especially if they are under 50. Among those younger women, the breast cancer death rate in blacks is 11 per 100,000, compared with only 6.3 in whites. A higher prevalence of basal-like breast tumors and a lower prevalence of luminal A tumors could contribute to the poor prognosis of young African American women with breast cancer. The finding has no immediate effect on treatment, because there is no treatment that specifically concentrates on basal-like cancer. Basal-like tumors tend to grow fast and spread quickly, and they are more likely than other types to be fatal. They are not estrogen-dependent, and cannot be treated or prevented with estrogen-blocking drugs like tamoxifen or raloxifene. Herceptin, another breast cancer drug, is also useless against these tumors. But efforts are being made to create drugs that will zero in on it. The work involves finding drugs to block specific molecules that these tumors need to grow.

### RATHER Consortium to Study Personalized Approach to Breast Cancer

RATHER consortium (Rational Therapy for Breast Cancer: Individualized Treatment for Difficult-to-Treat Breast Cancer Subtypes) investigators in Europe received a grant of €6 million ($8.4 million) from the EU in 2011 for a 5-year project to study two breast cancer types that are difficult to treat and amount to one quarter of all breast cancer cases: invasive lobular carcinoma and triple-negative breast cancer. The aim is to profile tumors in search of genetic mutations or other anomalous molecular processes involving kinases in these two cancer types in the hope that these studies will result in differences that are at the root of these cancers and could be targeted by novel drugs. RATHER partners include: University College Dublin; Agendia; Oncomark; the Netherlands Cancer Institute; the University of Cambridge;

the Curie Institute; Vall d'Hebron Institute of Technology; and Lund University. The partners plan to harmonize their methods to make comparisons between findings from each organization, and have started a project database that will be made available to the public. The partners also are working in collaboration with the European Bioinformatics Institute through shared participation in the EUROCANPLATFORM, which is also funded from the EU and will create a resource for use by the European scientific community.

**TAILORx (Trial Assigning Individualized Options for Treatment)**

Hormone therapy alone is usually given to women at low risk for recurrence of breast cancer and chemotherapy followed by hormonal therapy to women at a high risk for recurrence but there is uncertainty about the best way to handle cases that fall between low and high risk. There is need for a method of tailoring follow-up treatment that addresses the specific characteristics of a patient's tumor to enable an accurate prediction of what medical treatments will be most effective for long-term alleviation of the disease.

The TAILORx study is designed to examine whether women with early-stage lymph node-negative breast cancer can be assigned to individualized treatment plans based on certain genes that may predict whether their cancer will recur. It is sponsored by the NCI and will be conducted by all of the NCI-sponsored clinical trials groups that perform breast cancer research studies. TAILORx seeks to identify women who would not benefit from chemotherapy in order to spare them unnecessary treatment. The study is enrolling >10,000 women from 900 sites in the US and Canada. Women recently diagnosed with ER positive, Her2/neu-negative breast cancer, which has not yet spread to the lymph nodes, are eligible for the study. Using Oncotype DX™ (panel of 21 genes with known links to breast cancer), a modern diagnostic test developed by Genomic Health Inc in collaboration with the National Surgical Adjuvant Breast and Bowel Project, a network of cancer research professionals, TAILORx will determine the most effective cancer treatment, with the fewest side effects, for women with early-stage breast cancer. TAILORx is the first trial to be launched as part of a new NCI program – the Program for the Assessment of Clinical Cancer Tests (PACCT) – which seeks to individualize cancer treatment by using, evaluating and improving the latest diagnostic tests.

One TAILORx phase III clinical trial at uses genetic tests to obtain an individualized and quantitative analysis of how likely a specific patient's breast cancer is to recur. When a patient enrolls in the trial, a tumor tissue sample is sent to a central processing laboratory for Oncotype DX™ analysis. Using a statistical risk prediction model, a score is calculated that represents the specific patient's risk for breast cancer recurrence. The score is determined from the gene expression results using a range of zero to 100. Scores between 11 and 25 are considered to be in the intermediate or unclear risk category this trial focuses on. The information gathered from the genetic breast cancer test could give physicians a better understanding of the specific characteristics of their patients' breast tumors, which is critical in planning accurate treatment plans and follow-up.

### Tamoxin Therapy for ER-Positive Breast Cancer

For women with ER-positive early breast cancer, treatment with tamoxifen for 5 years substantially reduces the breast cancer mortality rate throughout the first 15 years after diagnosis. In the worldwide Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial, women with early breast cancer who had completed 5 years of treatment with tamoxifen were randomly allocated to continue tamoxifen to 10 years or stop at 5 years in the control group (Davies et al. 2013). The results showed that for women with ER-positive disease, continuing tamoxifen to 10 years rather than stopping at 5 years produces a further reduction in recurrence and mortality, particularly after 10 years. These results, taken together with results from previous trials of 5 years of tamoxifen treatment versus none, suggest that 10 years of tamoxifen treatment can reduce breast cancer mortality during the second decade after diagnosis.

### Triple Negative Breast Cancer

Subtypes of breast cancer are generally diagnosed based upon the presence or lack of three receptors that are known to fuel most breast cancers: progesterone receptors (PR), estrogen receptor (ER), and HER2. The most successful treatments for breast cancer target these receptors. Unfortunately, none of these receptors are found in women with triple negative breast cancer (TNBC), i.e. the offending tumor is ER-negative, PR-negative and HER2-negative. Therefore, TNBCs generally do not respond to receptor targeted treatments. However, this type of breast cancer is typically responsive to chemotherapy. Depending on the stage of its diagnosis, TNBC can be particularly aggressive, and more likely to recur than other subtypes of breast cancer. Metastatic TNBC (mTNBC) has a poor prognosis with median survival of 1 year as 30 % of patients suffer a recurrence after first line treatment. Causative BRCA1 mutations were detected in 9 % of TNBC patients, including patients without significant family histories and/or diagnosed at a later age (Rummel et al. 2013). The mutation frequency in patients <60 years was 11.2–18.3 % in those patients with significant risk factors and 4.6 % in those without, while in patients >60 years, the mutation frequency was 3.5–7.7 % in patients with risk factors, 2.3 % in those without. Thus, evaluation of additional risk factors in both patients younger and older than 60 years should improve the identification of TNBC patients benefiting from genetic testing of BRCA1.

Whole genome sequencing (WGS) has revealed previously unreported mutations in metastatic TNBC. Somatic genomic alterations in these advanced tumors, particularly those that might guide targeted therapies, have been cataloged following initial analyses of WGS and transcriptome sequencing data from prospective metastatic mTNBC (Craig et al. 2012). In a sample of 14 tumors from ethnically diverse metastatic TNBC patients, the researchers found significant mutations and other changes in more than a dozen genes through WGS performed on Life Technologies' SOLiD™ 4.0. The most frequently mutated gene among the tumors

(7 of 14) was the TP53 tumor suppressor, and aberrations were observed in additional tumor suppressor genes including CTNNA1, which was detected in 2 of 6 African-American patients (who typically have more aggressive and treatment-resistant disease). Alterations were also seen in the ERBB4 gene, known to be involved in mammary-gland maturation during pregnancy and lactation, but not previously linked to mTNBC. RNA sequencing revealed consistent overexpression of the FOXM1 gene, when tumor gene expression was compared to nonmalignant breast samples. Using an outlier analysis of gene expression comparing one cancer to all the others, the authors detected expression patterns unique to each patient's tumor. Integrative DNA/RNA analysis provided evidence for deregulation of mutated genes. Finally, molecular alterations in several cancers supported targeted therapeutic intervention on clinical trials with known inhibitors, particularly for alterations in the RAS/RAF/MEK/ERK and PI3K/AKT/MTOR pathways. In conclusion, whole genome and transcriptome profiling of mTNBC have provided insights into somatic events occurring in this difficult to treat cancer. These genomic data have guided patients to investigational treatment trials and provide hypotheses for future trials in this irremediable cancer. Genome sequencing will eventually become a standard tool for oncologists, enabling them to tailor therapies to the unique genetic profiles of each of their patients.

### Trends and Future Prospects of Breast Cancer Research

Currently expression profiling can uncover pathway regulation of gene expression and define molecular classes on the basis of integration of the total signals experienced by the cancer cell. The future trends that will have a great impact on breast cancer research are as follows:

- The data content will increase. Inclusion of miRNAs that are not well covered by the existing array technologies would result in greater precision and comprehensiveness.
- The analytical systems will become more informative.
- Metadata sets will emerge that will markedly expand the ability to validate and to model transcriptional networks of biological and clinical significance. This is already taking place with Oncomine and follows the success of other genomic databases. In molecular epidemiology, whole-genome SNP databases with linked clinical data are being made available to qualified researchers for analysis and data mining.

Primary breast cancers have been analyzed by genomic DNA copy number arrays, DNA methylation, exome sequencing, mRNA arrays, miRNA sequencing and reverse-phase protein arrays (Koboldt et al. 2012). Integration of information across platforms provided key insights into previously defined gene expression subtypes and demonstrated the existence of four main breast cancer classes when combining data from five platforms, each of which shows significant molecular heterogeneity. Somatic mutations in only three genes (TP53, PIK3CA and GATA3)

occurred at >10 % incidence across all breast cancers; however, there were numerous subtype-associated and novel gene mutations including the enrichment of specific mutations in GATA3, PIK3CA and MAP3K1 with the luminal A subtype. Two novel protein-expression-defined subgroups were identified, possibly produced by stromal/microenvironmental elements, and integrated analyses identified specific signaling pathways dominant in each molecular subtype including a HER2/phosphorylated HER2/EGFR/phosphorylated EGFR signature within the HER2-enriched expression subtype. Comparison of basal-like breast tumors with high-grade serous ovarian tumors showed many molecular features common to both cancers, indicating a related etiology and similar therapeutic opportunities. The biological finding of the four main breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities leads to the hypothesis that much of the clinically observable plasticity and heterogeneity occurs within, and not across, these major biological subtypes of breast cancer. This is the road map for how breast cancer might be cured in the future. Even within the four major types of breast cancer, individual tumors appear to be driven by their own sets of genetic changes. A wide variety of drugs will most likely need to be developed to be specifically effective for individual tumors. For example, PARP inhibitors that seem to be effective against ovarian cancers, may also be tried in basal-like breast cancer, which are most prevalent in younger women, in African-Americans and in women with breast cancer genes BRCA1 and BRCA2. Two other types of breast cancer, accounting for most cases of the disease, arise from the luminal cells that line milk ducts. These cancers have proteins on their surfaces that grab estrogen, fueling their growth. Genetic analysis divided these cancers into two distinct types. Patients with luminal A cancer had good prognoses while those with luminal B did not, suggesting that perhaps patients with the first kind of tumor might do well with just hormonal therapy to block estrogen from spurring their cancers while those with the second kind might do better with chemotherapy in addition to hormonal therapy. In some cases, genetic aberrations were so strongly associated with one or the other luminal subtype that they appeared to be the actual cause of the cancer. After basal-like cancers, and luminal A and B cancers, the fourth type of breast cancer frequently has extra copies of HER2 gene that drives their growth. Herceptin, can block the gene and has changed the prognosis for these patients from one of the worst in breast cancer to one of the best. Although Herceptin is approved for HER2 positive breast cancer patients, the new analysis finds that not all of these tumors are alike in responding to it. This is being investigated in further clinical trials.

This study demonstrates benefits of integrating genomic and proteomic data, particularly phosphoproteomics, which provided information beyond what the gene expression could. Proteomic data suggests the existence of two distinct phosphoproteomic-based subtypes within the larger gene expression-based HER2 subtype – one exhibiting high HER2 and HER1 signaling activity and the other exhibiting lower levels of such activity. The other example where the protein made one think was the analysis of PI3 kinase signaling, in which a disconnect was found between the PI3K signaling data obtained via the reverse-phase protein arrays analysis and their PI3K mutation data. A pathway-based analysis of the PI3K signaling

pathway revealed that what are believed to be protein and phosphoproteomic signatures of PI3K activation did not correlate with PI3K mutations, but did correlate with the loss of negative regulators of that pathway, like loss of INPP4B or loss of PTEN. Thus there is a discrepancy between the information from mutation and phosphoproteomics. The challenge now is to figure out which of these many different genetic events or protein signatures are going to be biomarkers of responsiveness to drugs like PI3K or mTOR inhibitors. This work is ongoing and the researchers are currently reanalyzing the genetic data based upon protein and phosphoproteomic endpoints.

**Understanding Tumor Diversity in Mouse Mammary Cancer Model**

Using a finding that the genetic complexity of tumors in mice parallels that in humans, researchers are conducting trial studies in mice similar to human clinical trials to evaluate whether an understanding of tumor diversity can improve cancer treatment. Analysis of tumors arising in the MMTV-Myc model of mammary carcinogenesis reveals substantial heterogeneity, seen in both histological and expression phenotypes (Andrechek et al. 2009). One of the MMTV-Myc mammary tumor subgroups exhibits metastatic capacity and that the signature derived from the subgroup can predict metastatic potential of human breast cancer. Together, these data reveal that a combination of histological and genomic analyses can uncover substantial heterogeneity in mammary tumor formation and therefore highlight aspects of tumor phenotype not evident in the population as a whole.

## *Personalized Management of Ovarian Cancer*

Epithelial ovarian carcinoma (EOC) is the most important cause of gynecological cancer-related mortality in Western societies. High-grade serous ovarian carcinoma (HGS-OvCa) accounts for 60–80 % of the ~35,000 women diagnosed with EOC in the US and ~40,000 in Europe annually. Known risk determinants for the development of ovarian carcinoma include BRCA1/BRCA2 mutations, family history, nulliparity, oral contraceptive use, tubal ligation, pregnancy, and lactation. A common treatment regimen consists of tumor debulking, followed by administration of platinum and taxane-based chemotherapy. Due to presentation of disease at advanced stages and development of resistance to therapy, the 5-year survival rate is <40 %. Early diagnosis, identification of nonresponders and patients with primary platinum resistance is an important step toward achieving greater life expectancy for EOC patients. Gene expression profiles have been established that are associated with overall survival and response to platinum therapy. Despite those encouraging developments, no biomarkers for prediction of response to therapy are yet in clinical use. New approaches for early diagnosis as well as treatment are, therefore, required to improve outcome in this disease.

### Early Diagnosis of Ovarian Cancer

Two tumor biomarkers, CA125 and one approved by FDA called HE4, are used to track whether chemotherapy is working or cancer is recurring. A one-time CA125 test cannot screen seemingly healthy women because levels rise with benign cysts, endometriosis, even normal menstruation, but Fujirebio's triage test uses HE4 and CA125 to assess who most likely has a benign cyst and whose has cancer.

OVA1® (Vermillion Inc) is a simple blood test cleared by the FDA to help physicians assess the likelihood that an ovarian mass is malignant prior to a planned surgery. OVA1® is not a screening test, but it is to be used together with a physician's overall assessment. OVA1® measures the levels of five proteins found in the blood and then uses proprietary software called OvaCalc® to calculate a single score. A woman's risk of cancer is measured by using a 0–10 scale versus predetermined cut-off points. Women who are pre-menopausal have a cut off score of 5 whereas postmenopausal women have a 4.4 cutoff. A high OVA1® score is not a diagnosis of cancer, rather it indicates an increased risk. An elevated OVA1® test score helps the physician to decide on referral to a gynecologic oncologist and increases the likelihood of optimal surgery, treatment and follow up for ovarian cancer patients.

A prospective, multi-institutional trial enrolled female patients scheduled to undergo surgery for an adnexal mass (Bristow et al. 2013). The multivariate index assay correctly identified 83.3 % malignancies missed by clinical impression and 70.8 % cases missed by CA125-II. Multivariate index assay was superior in predicting the absence of an ovarian malignancy, with a negative predictive value of 98.1 %. The study concluded that multivariate index assay demonstrated higher sensitivity and negative predictive value for ovarian malignancy compared to clinical impression and CA125-II in an intended-use population of non-gynecologic oncology practices.

### Determining Response to Chemotherapy in Ovarian Cancer

Gene expression profiles can predict response of ovarian cancer patients to chemotherapy. The method may enable clinicians to identify patients who are candidates for additional and/or novel chemotherapy drugs, and effectively choose appropriate cancer treatment. A variant in the 3′UTR of the KRAS oncogene, referred to as the KRAS variant, is associated with both cancer risk and altered tumor biology. KRAS variant can act as a biomarker of outcome in epithelial ovarian cancer (EOC). A study has shown that postmenopausal EOC patients with the KRAS variant are significantly more likely to die of ovarian cancer by multivariate analysis and are significantly more likely to be platinum resistant (Ratner et al. 2012). In addition, direct targeting of the KRAS variant leads to a significant reduction in EOC cell growth and survival in vitro. These findings confirm the importance of the KRAS variant in EOC, and indicate that the KRAS variant is a biomarker of poor outcome in EOC likely due to platinum resistance. In addition, this study supports the

hypothesis that these tumors have continued dependence on such 3′UTR lesions, and that direct targeting may be a viable future treatment approach.

Poly(ADP-ribose)polymerase (PARP) inhibitors have shown promising activity in patients with BRCA1/2 mutation-associated ovarian and breast cancers. Olaparib (AstraZeneca), a PARP inhibitor is, in two phase III trials for ovarian cancer. Understanding more about the molecular abnormalities involved in BRCA-like tumors, exploring novel therapeutic trial strategies and drug combinations, and defining potential predictive biomarkers, is critical to rapidly advancing the field of PARP inhibitor therapy and improve clinical outcomes (Lee et al. 2014). BRACAnalysis (Myriad Genetics) is being used as a companion test for olaparib to pick out ovarian cancer patients who are best responders to olaparib.

**Prognosis of Ovarian Cancer Based on CLOVAR**

The Cancer Genome Atlas catalog has been used to develop a prognostic model of HGS-OvCa classification called "Classification of Ovarian Cancer" (CLOVAR), which is based on subtype and survival gene expression profiles (Verhaak et al. 2013). Rather than relying on the 193 genes previously used in the signature of survival, the researchers focused on a smaller set, choosing the 100 genes whose expression were the most or least indicative of good prognosis from the full 481 TCGA HGS-OvCa sample set. Similarly, the researchers then revamped the subtype gene expression signature by narrowing the initial list of 800 genes down to 100 genes. Applied to a validation set, this new survival signature could stratify HGS-OvCa samples into a good prognosis group and a poor prognosis group. The worst outcome group, accounting for 23 % of all cases, was associated with a median survival of 23 months and a platinum resistance rate of 63 %, versus a median survival of 46 months and platinum resistance rate of 23 % in other cases. Associating the outcome prediction model with BRCA1/BRCA2 mutation status, residual disease after surgery, and disease stage further optimized outcome classification. The results of this study suggest that combining LOVAR survival, immunoreactivity, and mesenchymal scores with BRCA1/BRCA2 mutation status provides optimal outcome predictions. The spectrum of outcomes observed in this study and their association with CLOVAR signatures suggests variations in underlying tumor biology. The association uncovered between the CLOVAR survival, immunoreactive, and mesenchymal gene signature scores suggests an active role for the stromal tumor microenvironment in the pathogenesis of HGS-OvCa and may indicate possible targets for cancer therapies. An improved understanding of ovarian carcinoma development may ultimately lead to more effective treatments. Prospective validation of the CLOVAR model in the context of additional prognostic variables such as BRCA mutation status, age, grade, and residual disease may provide a rationale for optimal combination of patient and treatment regimens. A prospective study would be most revealing when assessing the predictive capacities of the CLOVAR signatures in conjunction with other prognostic factors.

**Recurrent and Drug-Resistant Ovarian Cancer**

To identify the best treatment for recurrent ovarian cancer, researchers at Yale School of Medicine (Harford, CT) are studying a technology called the Yale apoptosis assay in combination with another technology called the ChemoFX assay, which could double the response rate to existing drugs. In patients with recurrent ovarian cancer, it is often difficult to select an effective treatment because the tumor develops resistance to many drugs. Currently, physicians select a drug and must wait about six months to see whether it is effective on a particular patient. These two new assays will take the guesswork out of cancer treatment. Yale apoptosis assay is based on a biological principle that when a drug is effective, it will induce apoptosis in the cancer cell. If the cancer cell is resistant to a drug, apoptosis does not occur. The ChemoFX assay will determine whether a drug stops tumor growth. Used together, both assays will distinguish drugs that can stop the growth of the tumor and/or kill the tumor. This was not possible before. The technology will be studied with various cancers, starting with ovarian cancer. Each assay will be evaluated independently and then in combination in a multicenter clinical trial. The Yale research team partnered with Helomics, which has licensed and markets the ChemoFX assay. A study in 2009 at Duke University showed that >50 % of physicians followed results of ChemoFx® in management of ovarian cancer and the results changed physician behavior. Use of ChemoFx® results in cost savings of $2,900–$8,100 per patient per round for primary or recurrent ovarian cancer cases over a six-cycle treatment period.

The high incidence of recurrence attributable to multidrug resistance and the multiple histologic phenotypes indicative of multipotency suggests a stem cell-like etiology of ovarian cancer. Breast cancer-resistance protein 1-expressing verapamil-sensitive side population cells were identified in human ovarian cancer cell lines and primary ascites cells from patients with ovarian cancer. In the future, individualized therapy must incorporate analysis of the stem cell-like subpopulation of ovarian cancer cells when designing therapeutic strategies for ovarian cancer patients.

High-grade serous cancer (HGSC), the most common subtype of ovarian cancer, often becomes resistant to chemotherapy, leading to poor patient outcomes. Intratumoral heterogeneity occurs in nearly all solid cancers, including ovarian cancer, contributing to the development of resistance mechanisms. Past studies have identified a handful of resistance-related mutations in ovarian cancer, but unidentified CNVs could contribute to this process as well. A study has examined the spatial and temporal genomic variation in HGSC using high-resolution Affymetrix SNP 6.0 arrays (Cowin et al. 2012). Multiple metastatic lesions from individual patients were analyzed along with 22 paired pretreatment and posttreatment samples. The authors documented regions of differential DNA copy number between multiple tumor biopsies that correlated with altered expression of genes involved in cell polarity and adhesion. In the paired primary and relapse cohort, they observed a greater degree of genomic change in tumors from patients that were initially sensitive to chemotherapy and had longer progression-free interval compared with tumors from patients that were resistant to primary chemotherapy. Notably, deletion

or downregulation of the lipid transporter LRP1B emerged as a significant correlate of acquired resistance in the analysis. Functional studies showed that reducing LRP1B expression was sufficient to reduce the sensitivity of HGSC cell lines to liposomal doxorubicin, but not to doxorubicin, whereas LRP1B overexpression was sufficient to increase sensitivity to liposomal doxorubicin. Together, these findings underscore the large degree of variation in DNA copy number in spatially and temporally separated tumors in HGSC patients. LRP1B is defined as a potential contributor to the emergence of chemotherapy resistance in these patients and may serve as a biomarker for such acquired resistance. Mapping the mechanisms that confer resistance may enable prediction of whether some women are likely to respond to a certain drug or not, and find ways of reversing resistance.

Quantitative RT-PCR of 3 miRs (miR-484, -642, and -217) involved in angiogenesis is able to predict chemoresistance of ovarian cancer (Vecchione et al. 2013). Additional analysis of miR-484 reveals that the sensitive phenotype is caused by a modulation of tumor vasculature through the regulation of the VEGFB and VEGFR2 pathways. These findings suggest that blockage of VEGF by use of an anti-VEGFA antibody may not be sufficient to improve survival in ovarian cancer patients unless VEGFB signaling is also blocked. Alternatively, small compounds, such as functionalized nanoparticles targeting the VEGFR1 and VEGFR2 receptors, could be used as effective therapy in these patients, changing the course of prognosis and treatment of ovarian cancer (Liu et al. 2011).

**Pathway Targeted Therapies for Ovarian Cancer**

Mouse ovarian epithelial tumor cell lines that contain various combinations of genetic alterations in the p53, c-myc, K-ras and Akt genes have been used as model for the molecular characterization of pathway-targeted therapy. Response to a particular anticancer drug can be related to the signaling pathway involved. Rapamycin effectively inhibits the growth of tumors that rely on Akt signaling for proliferation, whereas tumors in which Akt signaling is not the driving force in proliferation are resistant to rapamycin. The introduction of activated Akt to the rapamycin-resistant cells does not render the cells susceptible to rapamycin if they can use alternative pathways for survival and proliferation. Therefore, the rapamycin-sensitive tumors develop resistance to rapamycin when presented with alternative survival pathways, such as the mitogen-activated extracellular kinase signaling pathway. The combination of rapamycin and the mitogen-activated extracellular kinase inhibitor PD98059 is required to diminish proliferation in these cell lines. These findings indicate that mammalian target of rapamycin inhibitors may be effective in a subset of tumors that depend on Akt activity for survival but not effective in all tumors that exhibit Akt activation. Tumors with alternative survival pathways may require the inactivation of multiple individual pathways for successful treatment. These results have significant implications for the use of pathway-targeted therapy in advanced human ovarian cancers, which typically display numerous genetic alterations that are likely to require impairment of multiple molecular pathways for successful treatment.

Interruption of multiple specific biochemical pathways may be a promising therapeutic strategy in ovarian carcinomas that exhibit resistance to an individual targeted therapy. This strategy may be useful for developing personalized therapies for ovarian cancer.

Human ovarian cancer stem cells (CSCs) have been characterized and shown to have a distinctive genetic profile that confers them with the capacity to recapitulate the original tumor, proliferate with chemotherapy, and promote recurrence (Alvero et al. 2009). CSCs identified in ovarian cancer cells isolated form ascites and solid tumors are characterized by cytokine and chemokine production, high capacity for repair, chemoresistance to conventional chemotherapies, and resistance to TNFα-mediated apoptosis. Chemotherapy eliminates the bulk of the tumor but it leaves a core of CSCs with high capacity for repair and renewal. The molecular properties identified in these cells may explain some of the unique characteristics of CSCs that control self-renewal and drive metastasis. The identification and cloning of human ovarian CSCs can aid in the development of better therapeutic approaches for ovarian cancer patients.

Resistance to platinum therapy is a major obstacle that needs to be overcome in the treatment of ovarian cancer patients. The high rates and patterns of therapeutic failure seen in patients are consistent with a steady accumulation of drug-resistant CSCs. A study has demonstrated that the Notch signaling pathway and Notch3 in particular are critical for the regulation of CSCs and tumor resistance to platinum (McAuliffe et al. 2012). Notch3 overexpression in tumor cells results in expansion of CSCs and increased platinum chemoresistance. In contrast, γ-secretase inhibitor (GSI), a Notch pathway inhibitor, depletes CSCs and increases tumor sensitivity to platinum. Similarly, a Notch3 siRNA knockdown increases the response to platinum therapy, further demonstrating that modulation of tumor chemosensitivity by GSI is Notch specific. Most importantly, the cisplatin/GSI combination is the only treatment that effectively eliminates both CSCs and the bulk of tumor cells, indicating that a dual combination targeting both populations is needed for tumor eradication. In addition, cisplatin/GSI combination therapy has a synergistic cytotoxic effect in Notch-dependent tumor cells by enhancing the DNA-damage response, G2/M cell-cycle arrest, and apoptosis. These findings indicate that targeting the Notch pathway significantly increases tumor sensitivity to platinum therapy. Both platinum-resistant and platinum-sensitive relapses may benefit from such an approach as clinical data suggest that all relapses after platinum therapy are increasingly platinum resistant.

Two strategies for targeted therapy have emerged with promising results: poly ADP-ribose polymerase enzyme (PARP) inhibitors and targeting angiogenesis, but development of a convenient and accurate method to identify patients likely to benefit from these remains a challenge (Gómez-Raposo et al. 2011).

A catalogue of molecular aberrations that cause ovarian cancer is critical for developing and deploying therapies that will improve patients' lives. The Cancer Genome Atlas project has analyzed mRNA expression, miRNA expression, promoter methylation and DNA copy number in high-grade serous ovarian adenocarcinomas and the DNA sequences of exons from coding genes in most of these tumors

(The Cancer Genome Atlas Research Network 2011). The equipment used included Agilent, Illumina, and Affymetrix arrays to look at CNV, mRNA expression, miRNA expression, and methylation profiles of tumor samples. Whole-exome sequencing was carried out on a subset of these. High-grade serous ovarian cancer was found to be characterized by TP53 mutations in almost all tumors. Pathway analyses suggested that homologous recombination was defective in about half of the tumors analyzed, and that NOTCH and FOXM1 signaling are involved in serous ovarian cancer pathophysiology. Although relatively few genes were found to contain recurrent mutations in the ovarian cancer, the researchers tracked down numerous CNVs and several frequently mutated pathways, along with miRNA, methylation, and transcription signatures that hold promise for categorizing ovarian cancer and predicting survival outcomes. Overall, these discoveries set the stage for approaches to the treatment of high-grade serous ovarian cancer in which aberrant genes or networks are detected and targeted with therapies selected to be effective against these specific aberrations.

### Targeting Hematogenous Metastasis of Ovarian Cancer

Ovarian cancer has a clear predilection for metastasis to the omentum, but the underlying mechanisms involved in ovarian cancer spread were not well understood. A study used OncoCEE microfluidic device (Biocept Inc), which captures CTCs and then evaluates the expression of the genomic marker HER3 in ovarian cancer cells, demonstrated preferential hematogenous metastases of ovarian cancer to the omentum (Pradeep et al. 2014). The study revealed that the ErbB3-neuregulin 1 (NRG1) axis is a dominant pathway responsible for hematogenous omental metastases. Elevated levels of ErbB3 in ovarian cancer cells and NRG1 in the omentum have enabled tumor cell localization and growth in the omentum. Depletion of ErbB3 in ovarian cancer impaired omental metastases. These results highlight hematogenous spread as an important mode of ovarian cancer metastases and use of this knowledge to design better strategies for prevention and treatment.

### Vynfinit ® for Platinum-Resistant Ovarian Cancer

Platinum-resistant ovarian cancer (PROC) is a challenging disease with a high unmet need for new treatments. PROC recurs within 6 m of completion of a platinum-containing regimen, the standard of care for ovarian cancer. An estimated 80 % of platinum-resistant ovarian cancer patients have been found to have folate receptor-positive disease, and ~40 % express the receptor, as detected by etarfolatide, in all of their target tumor lesions. Compared to patients who do not express folate receptors on their tumors, folate receptor-positive patients have been shown to have a poorer overall prognosis.

Vintafolide is a conjugate of folic acid (vitamin B9) linked to an anticancer agent, the potent vinca alkaloid desacetylvinblastine hydrazide. Since cancer cells

generally consume higher levels of folate than normal cells to fuel their growth, some cancer cell types, including ovarian, have high concentrations of the folate receptor on their surface. Vintafolide is designed to selectively target the folate receptor to deliver the anti-cancer agent to the cancerous tissue. Tumors that have high concentrations of the folate receptor are identified by etarfolatide, a non-invasive imaging diagnostic agent. Intravenous folic acid is used with $^{99m}$Tc-etarfolatide for the enhancement of image quality.

EMA has approved vintafolide (Merck & Co/Endocyte's Vynfinit ®) and companion imaging agents, etarfolatide (Folcepri ®) as well as intravenous folic acid (Neocepri ®), in patients with platinum-resistant ovarian cancer. FDA has also granted orphan drug status to vintafolide and etarfolatide. Further evaluation is ongoing in the global PROCEED phase III clinical trial in folate receptor-positive, PROC.

## *Personalized Management of Head and Neck Cancer*

Molecular targeted therapy in head and neck squamous cell carcinoma (HNSCC) continues to make strides, and holds much promise. Cetuximab remains the sole FDA-approved molecular targeted therapy available for HNSCC, though several new biologic agents targeting the EGFR and other pathways are currently in the regulatory approval pipeline. While targeted therapies have the potential to be personalized, their current use in HNSCC is not personalized. This is illustrated for EGFR-targeted drugs, where EGFR as a molecular target has yet to be individualized for HNSCC. Future research needs to identify factors that correlate with response (or lack of one) and the underlying genotype-phenotype relationship that dictates this response. Comprehensive exploration of genetic and epigenetic landscapes in HNSCC is opening new frontiers to further enlighten and mechanistically inform newer as well as existing molecular targets, and to set a course for eventually translating these discoveries into therapies for patients. A snapshot of the evolution of molecular subtyping in HNSCC and its current clinical applicability, as well as new emergent paradigms with implications for controlling this disease in the future has been presented in an opinion article (Worsham et al. 2012).

### Relevance of Biomarkers of HPV-Related Head and Neck Cancer

Some reports have associated a subset of HNSCC with high-risk human papillomaviruses (HPVs), particularly HPV16, the same subset of HPVs responsible for the majority of cervical and anogenital cancers. A positive test for HPV DNA alone was not significantly linked to head and neck squamous cell carcinoma (HNSCC) outcomes. On the other hand, when found in combination with E6 and E7 expression, a positive HPV16 test did coincide with improved oropharyngeal cancer outcomes. Likewise, elevated levels of p16 in a tumor were not especially informative on their

own, though they did correspond to better oropharyngeal cancer survival when found together with positive blood tests for E6 and E7. Based on these findings, it is concluded that a stronger association of HPV presence with prognosis (assessed by all-cause survival) is observed when HPV-associated HNSCC is defined using tumor status (HPV DNA or P16) and HPV E6/E7 serology in combination rather than using tumor HPV status alone (Liang et al. 2012).

Another study on oropharyngeal squamous cell carcinomas (OPSCC) found its own evidence arguing against the use of HPV DNA as a solo biomarker for HPV-associated cancer (Holzinger et al. 2012). They authors tested OPSCC tumors for HPV DNA and p16. They also considered the viral load in the tumors and looked for gene expression profiles resembling those described in cervical carcinoma, another cancer associated with HPV infection. Again, the presence of HPV DNA appeared to be a poor indicator of HPV-associated cancers or predictor of cancer outcomes. Whereas nearly half of the tumors tested positive for HPV16 DNA, just 16 % and 20 % had high viral loads and cervical cancer-like expression profiles, respectively. The researchers found that a subset of HPV DNA-positive tumors with high viral load or HPV-associated expression patterns belonged to individuals with better outcomes. In particular, they found that cervical cancer-like expression profiles in OPSCC tumors coincided with the most favorable outcomes, while high viral load in the tumors came a close second. Once standardized assays for these biomarkers, applicable in routine clinical laboratories, are established, they will allow precise identification of patients with oropharyngeal cancer with or without HPV-driven cancers and, thus, will influence prognosis and potentially treatment decisions. More research is needed to understand whether the patterns described in the new studies hold in other populations and to tease apart the prognostic importance of HPV infection in relation to additional prognostic biomarkers.

## *Personalized Management of Hematological Malignancies*

Myeloproliferative disorders include several pathologies sharing the common feature of being clonal hematopoietic stem cell diseases. Hematological malignancies are highly heterogeneous in the matter of molecular mechanisms related to their development and progression. A considerable heterogeneity can be further observed within the same disease at the interindividual level as reflected by different clinical outcomes and responses to treatment in different patients. Considerable work has been done on molecular cytogenetics of hematological malignancies and a number of diagnostics and therapies are available or under development.

The molecular basis of chronic myeloid leukemia (CML) was characterized many years ago with the discovery of the t(9;22) translocation and its product the BCR-ABL oncoprotein. The finding of a recurrent mutation in the Janus 2 tyrosine kinase (JAK2) gene was a major advance in understanding of the pathogenesis of several other myeloproliferative disorders, including polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis. Such a recurrent and unique

mutation leading to a tyrosine kinase deregulation would make a suitable target for the development of specific therapies. QIAGEN Marseille has worldwide exclusive IP rights to a test based on mutations in the JAK2 gene.

The advent of high-throughput NGS technologies, which are revolutionizing genomics and transcriptomics by providing a single base resolution tool for a unified deep analysis of diseases complexity, enable fast and cost-efficient fine-scale assessment of the genetic variability hidden within cohorts of patients affected by the same leukemia. That being so, by potentially highlighting inter-individual differences that may play a role in the differential success of diverse therapeutic interventions, they promise to be crucial for selecting the most appropriate medical treatments.

The NGS-PTL (personalized therapy of leukemia) project aims at developing a European platform of scientists for improving outcomes for therapeutic interventions on acute and chronic leukemias and at developing strategies to personalize treatments and tailor therapies to different groups of leukemia patients, with the main goal of optimizing their efficacy and safety through a deeper understanding of the influence of genomic alterations on leukemias pathogenesis and treatment response. The systematic whole exome/transcriptome studies on clinically well-characterized leukemia patients scheduled within the project are therefore expected to help the identification of novel prognostic biomarkers for acute and chronic leukemias, as well as of molecular biomarkers and/or genome-wide profiles for the assessment of minimal residual disease.

## Personalized Management of Acute Lymphoblastic Leukemia

Progress in the molecular classification of acute lymphoblastic leukemia (ALL) with the use of DNA microarrays combined with methods to assess the functional significance of newly discovered genes or through proteomic techniques, will lead to the identification of targets for specific treatments. Imatinib mesylate, introduced for the treatment of BCR-ABL-positive CML, inhibits the BCR-ABL fusion protein and other constitutively active tyrosine kinases and induces transient remissions of BCR-ABL-positive ALL as well as partial responses in other cancers, is the forerunner of a new generation of molecularly targeted anticancer drugs. Other potentially useful agents that are under development include inhibitors of FLT-3 tyrosine kinases for use against leukemias characterized by activating mutations of this kinase and inhibitors of histone deacetylase for leukemias such as TEL-AML1-positive ALL. Further refinements in the molecular classification of ALL, together with the identification of genetic features that affect the efficacy and toxicity of antileukemic therapy, will provide unique opportunities to devise treatment plans for individual patients and thus to realize the elusive goal of cure in all patients, regardless of their presenting characteristics. ALL is treated with a cocktail of chemotherapeutic agents that include 6-mercaptopurine, 6-thioguanine and azathiopurine. These drugs are broken down by the (TPMT). Those lacking functional TPMT can suffer severe toxicity or death but these patients can be treated with doses that

are much lower than the standard regimen. Physicians at St. Jude's Children's Hospital (Memphis, TN) and at the Mayo Clinic (Rochester, MN) are prescreening patients to determine if they have functional or nonfunctional enzyme thiopurine methyl transferase (TPMT). The dosage of the components in the chemotherapeutic cocktail are then tailored precisely to the patient's molecular makeup – personalized prescribing. TPMT genotype also has a substantial impact on minimal residual disease (MRD) after administration of mercaptopurine in the early course of childhood ALL, most likely through modulation of mercaptopurine dose intensity. These findings support a role for MRD analyses in the assessment of genotype-phenotype associations in multiagent chemotherapeutic trials. Investigators at St. Jude Children's Research Hospital have also developed a relatively simple and inexpensive test that identifies children with ALL who have responded well enough to their first round of chemotherapy that they might be successfully treated with a much less aggressive follow-up treatment.

Genetic variation in the enzymes of the folic acid cycle, one-carbon transfer, immune surveillance, drug metabolism and transport may determine some of the variability in treatment response of ALL patients. Despite recent advances in this area, further work is needed to develop clinically useful genetic predictors of leukemia treatment response (Cunningham and Aplenc 2007).

Risk factors for CNS relapse in childhood ALL included the genetic abnormality t(1;19)(TCF3-PBX1), any CNS involvement at diagnosis, and T-cell immunophenotype. At St. Jude Children's Hospital, personalized therapy is applied based on molecular genetics of ALL, pharmacogenetic traits of patients and pharmacodynamic principles. The activity of drug-metabolizing enzymes of each patient is determined prospectively and the dosage of chemotherapy is adjusted accordingly. It was demonstrated that with effective risk-adjusted personalized chemotherapy, prophylactic cranial irradiation can be safely omitted from the treatment of childhood ALL (Pui et al. 2009). This chemotherapy approach produced a projected cure rate of 90 % for all the patients, which is the best treatment result reported to date.

**Personalized Management of Acute Myeloid Leukemia**

Two molecular tests for acute myeloid leukemia (AML) from Genzyme Diagnostics are relevant to personalized management: FLT3 Mutation Analysis and WT1 RQ-PCR. FLT3 mutations are considered a prognostic indicator of poor survival and response to standard chemotherapies. Approximately 30 % of patients with AML have FLT3 mutations. WT1 RQ-PCR test is designed to detect MRD or very low levels of disease. The WT1 gene is expressed in approximately 90 % of patients with AML. This test allows physicians to monitor AML patients for early relapse during and following therapy. Both of these tests may enable oncologists to better manage their patients. Laboratory for Personalized Molecular Medicine is developing a companion diagnostic for the identification of FLT3-positive AML patients for treatment with Novartis' midostaurin, or PKC412, a targeted small molecule inhibitor of FLT3 tyrosine kinase, which is currently in phase III clinical trials.

American Society of Clinical Oncology and the National Comprehensive Cancer Network recommend testing for the FLT3 mutation, and determination of FLT3 status as a standard of care for patients diagnosed with AML.

Activating internal tandem duplication (ITD) mutations in FLT3 (FLT3-ITD) are associated with a poor prognosis. Scientific evidence including the lack of convincing clinical activity of early FLT3 inhibitors suggests that FLT3-ITD probably represents a passenger lesion. Point mutations have been reported at three residues within the kinase domain of FLT3-ITD that confer substantial in vitro resistance in AML patients to AC220 (quizartinib), an active investigational inhibitor of FLT3 (Smith et al. 2012). These findings demonstrate that FLT3-ITD can represent a driver lesion and valid therapeutic target in human AML. AC220-resistant FLT3 kinase domain mutants represent high-value targets for future FLT3 inhibitor development efforts.

Risk stratification in AML is currently based on pretreatment characteristics. It remains to be established whether relapse risk can be better predicted through assessment of minimal residual disease (MRD). One proposed marker is the Wilms tumor gene WT1, which is overexpressed in most patients with AML, thus providing a putative target for immunotherapy. An international collaborative study coordinated by the European Leukemia Network (ELN) consortium on standardization of WT1 testing for risk stratification in AML has been published (Cilloni et al. 2009). The objective of this study was to select the best-performing WT1 assay and to assess the value of WT1 monitoring during AML treatment to estimate the risk of relapse. Results of the study show that the high performance WT1 assay designed by the ELN group is adapted to MRD assessment. This specific assay developed and validated in the context of this study, WT1 ProfileQuant® (QIAGEN Marseille) is CE marked and can be used with most RQ-PCR instruments. Application of a standardized WT1 assay provides independent prognostic information in AML, lending support to incorporation of early assessment of MRD to develop more robust risk scores, to enhance risk stratification, and to identify patients who may benefit from allogeneic transplantation.

Cytarabine (ara-C) is the most effective agent for the treatment of childhood AML but aberrant expression of enzymes involved in the transport/metabolism of ara-C could explain drug resistance. Human equilibrative nucleoside transporter-1 (hENT1) mRNA expression and ara-C sensitivity have been correlated with threefold lower hENT1 mRNA levels in resistant patients. Thus decreased expression of hENT1, which transports ara-C across the cell membrane, is a major factor in ara-C resistance in childhood AML. In 2011, Clavis Pharma received a Norwegian government grant to develop a flow cytometry method for the detection and quantification of human hENT1 in patients suffering from AML, which will enable the selection of the sub-population of AML patients who are likely to benefit most from treatment with the novel anticancer drug elacytarabine.

Because response of AML patient to cytarabine-based standard-of-care treatment is variable, stratification into subgroups by biomarker-predicted response may lead to improved clinical outcomes. Cell mitochondrial depolarization to proapoptotic signaling BH3-only peptides has been assessed as a surrogate for the function

of Bcl-2 family proteins to address clinical response to cytarabine-based therapy in patients with AML (Pierceall et al. 2013). Peripheral blood mononuclear cell or bone marrow aspirate specimens were obtained from newly diagnosed patients with AML, viably preserved, and assayed by flow cytometry following BH3 profile assay with individual BH3 peptides. When patients were stratified by cytogenetic status, readout was significant for both intermediate and unfavorable risk groups, demonstrating predictive power independent of cytogenetics. Additional analyses of secondary clinical endpoints displayed correlation between overall survival and event-free survival when patients were stratified by peptide response. Taken together, these results highlight the potential utility of BH3 profiling in personalized diagnostics of AML by offering actionable information for patient management decisions. Eutropics Pharmaceuticals' PraediCare technology to develop a companion diagnostic for leukemia is based on BH3 profiling.

Several mutations that contribute to the pathogenesis of AML remain undefined and the relationships between patterns of mutations and epigenetic phenotypes continue to be investigated to improve an understanding of pathomechanism of AML as a basis for personalized management. Genomes of 200 clinically annotated adult cases of de novo AML have been analyzed, using either WGS (50 cases) or WES (150 cases), along with RNA and miRNA sequencing and DNA-methylation analysis (The Cancer Genome Atlas Research Network 2013). Results show that AML genomes have fewer mutations than most other adult cancers. At least one potential driver mutation was identified in nearly all AML samples. This mutation was in one of nine categories of genes that are almost certainly relevant for pathogenesis, including transcription-factor fusions the gene encoding nucleophosmin, tumor-suppressor genes, DNA-methylation-related genes, signaling genes, chromatin-modifying genes, myeloid transcription-factor genes, cohesin-complex genes, and spliceosome-complex genes. A complex interplay of genetic events was found to contribute to AML pathogenesis in individual patients. Integrated of the expression data for both mRNA and miRNA with all the clinical and mutational data for all genomes in this study revealed that the differentiation state of the AML samples was highly correlated with the expression signature, as reported previously. Patients who had PML-RARA fusions had very distinct mRNA and miRNA signatures that were strongly correlated with each other and with a specific DNA methylation signature. All the transcription factor fusions were correlated with specific patterns of mRNA expression, whereas PML-RARA and RUNX1-RUNX1T1 (and some MLL fusions) were also associated with miRNA expression signatures. In addition, occurrence of NPM1, DNMT3A, and FLT3 mutations together was strongly associated with specific expression signatures for both mRNA and miRNA. These data suggest that this combination of mutations in patients with intermediate-risk AML may identify a subtype of AML with unique epigenetic features. The databases from this study are widely available to serve as a foundation for further investigations of AML pathogenesis, classification, and risk stratification.

**Personalized Management of Chronic Lymphocytic Leukemia**

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world with the majority of cases occurring in patients over the age of 55. It usually progresses slowly and is characterized by the accumulation of lymphocytes, which can overwhelm the bone marrow and invade the blood stream, eventually spreading to the spleen, liver and other solid organs. CLL, however, has a highly variable clinical course: slowly progressive in some, whereas others have an aggressive disease. In the last quarter of twentieth century, prognosis and treatment decisions were based on clinical staging systems. In the twenty-first century, biomarkers have enabled a more refined prognostic stratification. In spite of advances in whole genome sequencing, CLL is not associated with a specific genetic abnormality. However, B cell receptor signaling, which may be constitutively expressed, antigen-induced, or both, plays a critical role in driving cell proliferation in CLL and their survival through the cascade of protein kinases. Elimination of CLL to an extremely low level may improve the overall and treatment-free survival. Patients with no detectable CLL cells after receiving Campath (alemtuzumab) usually survive for >5 years. CLL patients who relapse from or are refractory to chemotherapy have the poorest prognosis with a median survival of 10 m. A test to detect MRD in patients with B cell CLL to complement the treatment with Campath® is an example of combining diagnostics with therapy to improve the treatment.

The pathognomonic genetic alteration in CML is the formation of the BCR-ABL1 fusion gene, which produces a constitutively active tyrosine kinase (TK) that drives leukemic transformation. Targeted TK inhibitor treatment with imatinib, nilotinib, dasatinib, bosutinib, and ponatinib is the cornerstone of modern therapy for this hematologic malignancy. Ibrutinib, an oral inhibitor, has shown activity in a small series of patients with relapsed or refractory CLL or small lymphocytic lymphoma (Advani et al. 2013). In a phase Ib clinical trial, ibrutinib was associated with a high frequency of durable remissions in patients with relapsed or refractory CLL and small lymphocytic lymphoma, including patients with high-risk genetic lesions (Byrd et al. 2013). This is an example of trend in management of hematologic cancers, which is shifting from a chemotherapy-based approach to treatments aimed at mechanisms of disease. New prognostic subgroups in CLL based on integrated mutational and cytogenetic analysis are (Rossi et al. 2013):

1. High risk (10-years survival <30 %): TP53 abnormalities, BIRC3 abnormalities, or both.
2. Intermediate risk: NOTCH1 mutations, SF3B1 mutations, or both, with or without 11q22.3 deletion.
3. Low risk: trisomy 12 or normal cytogenetic profile.
4. Very low risk (10-years survival ~70 %): 13q14 deletion only.

RT-qPCR of BCR-ABL1 RNA is a necessary laboratory technique for monitoring the efficacy of TK inhibitor therapy and quantitatively assessing MRD. The molecular response measured by BCR-ABL1 RT-qPCR assists in identifying suboptimal responses and can help the decision to switch to alternative therapies that

may be more efficacious. Furthermore, TK inhibitor-mediated molecular response provides valuable risk stratification and prognostic information on long-term outcomes. Despite these attributes, informed, universal, practical utilization of this well-established monitoring test will require heightened efforts by the molecular diagnostics laboratory community to adopt the standardized reporting units of the International Scale. Without widespread adoption of the International Scale, the consensus major molecular response and early molecular response treatment thresholds will not be definable, and optimal clinical outcomes for patients with CML may not be achieved (Press et al. 2013).

**Personalized Management of Multiple Myeloma**

Multiple myeloma (MM), the second most common hematological cancer after non-Hodgkin's lymphoma, is considered incurable although some patients survive for a number of years following diagnosis. About 50,000 people in the US are living with the disease, and an estimated 16,000 new cases are diagnosed annually. Despite improvements in therapy, the 5-year survival rate in multiple myeloma is only 32 % and durable responses are rare. Multiple myeloma is a neoplasia of clonally expanded malignant bone marrow plasma cells. Previously two genetic subtypes of myeloma were known: (1) hyperdiploid MM characterized by extra copies of entire chromosomes and patients with this subtype appear to fare better; (2) non-hyperdiploid form lacks these extra chromosomes and instead has abnormal rearrangements between different chromosomes with worse outlook for the patients with this subtype. The roles played by various abnormalities in the initiation and progression of myeloma are only beginning to be understood, but it been observed that different abnormalities vary from one patient to the other.

Pharmacogenomic studies in multiple myeloma are helping to set the stage for individualized therapy. Although relatively few in numbers, these studies are already providing new therapeutic targets and avenues for drug discoveries as well as contributing to novel prognostic markers in multiple myeloma. Genetics and gene expression profiling technology have improved molecular-based patient stratification and prognostic staging, expanded knowledge of the molecular mechanism of chemotherapeutic agents, and provided a better understanding of multiple myeloma.

Distinct genetic subtypes of MM have different prognoses and might be treated most effectively with drugs specifically targeted to those subtypes. For further analysis of many DNA alterations in the MM genome, an algorithm has been created based on a computational method, which is used to group the results in a way that yield distinctive genomic features from the CGH data. Four distinct myeloma subtypes based on genetic patterns emerge from these data of which two correspond to the non-hyperdiploid and hyperdiploid types; the latter contains two further subdivisions, called k1 and k2. Those with the k1 pattern have a longer survival than those with k2. These results define new disease subgroups of MM that can be correlated with different clinical outcomes. The findings pave the way for treat-

ments tailored to a patient's specific form of the disease and also narrow down areas of the chromosomes in myeloma cells likely to contain undiscovered genetic aberrations that drive myeloma, and which might turn out to be vulnerable to targeted designer drugs.

Researchers at Mayo Clinic Cancer Center, in cooperation with industry partners, have identified tumor specific alterations in the cellular pathway by which the MM drug bortezomib (Velcade) works and they have identified nine new genetic mutations in cancer cells that should increase a patient's chance of responding to the agent. These findings, may help physicians tailor treatment to patients with MM. Bortezomib seems to work in about one-third of patients who use it, but up to now it was not possible to predict which ones. Investigators have identified a group that will likely respond because these nine mutations seem to be present in at least 25 % of newly diagnosed patients. Multiple genetic mutations in the other NF-κB pathway, the so-called non-canonical pathway, make the tumor more dependent on that pathway, and consequently more susceptible to bortezomib treatment. Identifying these mutations in patients will help the decision as to which patients should be treated with bortezomib, probably as an initial therapy. A test is in development to check for activation of the non-canonical NF-κB pathway in patients. Now that the mutations have been identified, drug designers may be able to fashion new therapies that are more specific to these genetic alterations and, therefore, less toxic. These mutations represent good targets for drug development.

Despite overwhelming genomic chaos in multiple myeloma (MM), expression patterns within tumor samples are remarkably stable and reproducible. Unique expression patterns associated with recurrent chromosomal translocations and ploidy changes defined molecular classes with differing clinical features and outcomes. Combined molecular techniques also dissected two distinct, reproducible forms of hyperdiploid disease and have molecularly defined MM with high risk for poor clinical outcome. Gene-expression profiling (GEP) is now used to risk-stratify patients with newly diagnosed MM. Groups with high-risk features are evident in all GEP-defined MM classes, and GEP studies of serial samples showed that risk increases over time, with relapsed disease showing dramatic GEP shifts toward a signature of poor outcomes. This suggests a common mechanism of disease evolution and potentially reflects preferential expansion of therapy-resistant cells. Correlating GEP-defined disease class and risk with outcomes of therapeutic regimens reveals class-specific benefits for individual agents, as well as mechanistic insights into drug sensitivity and resistance (Zhou et al. 2009).

Signal Genetics' scientists have analyzed the expression levels of thousands of human genes that are considered to be linked to MM. Clustering analysis of these various genes gave rise to the 70 most relevant myeloma linked genes used to make up the patient's gene expression profile, which is the basis of My Prognostic Risk Signature™ (MyPRS™) as a diagnostic supplement for MM, which can help to design a personalized regimen. The median survival rate for MM in the US is 2.5–3 years, but personalized approach can raise the median survival rate to 6–7 years.

### Personalized Management of Myelodysplastic Syndrome

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders of ineffective hematopoiesis that characteristically demonstrate peripheral blood cytopenia, bone marrow hypercellularity, and morphologically defined dysplasia of one or more hematopoietic lineages. Classical metaphase cytogenetics and judicious use of FISH play central roles in the contemporary diagnosis and classification of MDS. An abundance of recent molecular studies are beginning to delineate additional genetic and epigenetic aberrations associated with these disorders, which affect diagnosis, prognosis, and therapy. Classification systems are evolving from a primarily hematological and morphological basis toward a multifactorial appreciation that includes histomorphology, metaphase cytogenetics, and directed molecular studies. Rapidly growing understanding of the genetic basis of MDS holds much promise for testing, and a frame of reference has been provided for discussion of current testing protocols and for addressing testing modalities likely to enter clinical practice in the near future (Nybakken and Bagg 2014).

Cytogenetic analyses are mandatory for risk stratification and for monitoring response to drug treatment in MDS. Low-dose demethylating agents such as 5-aza-2′-deoxycytidine (decitabine) and 5-azacytidine (azacitidine, Vidaza) have been explored for the treatment of MDS with the aim to revert a methylator phenotype. Decitabine treatment is associated with a response rate that is higher in patients with high-risk cytogenetics (i.e., complex karyotype and/or abnormalities of chromosome 7) than in patients with intermediate-risk cytogenetics (two abnormalities or single abnormalities excluding 5q-, 20q-, and -Y). Following decitabine treatment of patients with abnormal karyotype, approximately one-third achieve a major cytogenetic response that can be confirmed by FISH analyses, while in two-thirds of patients, the abnormal karyotype persists but hematologic improvement may be observed during continued treatment. The most frequently studied gene in myelodysplasia is the cell cycle regulator p15. Hypermethylation of p15 in MDS is reversed during treatment with decitabine, resulting in reactivation of this gene.

Somatic mutations in MDS may influence the clinical phenotype but are not included in current prognostic scoring systems. Combination of genomic approaches, including NGS and MS-based genotyping, identified somatic mutations in 18 genes in samples of bone marrow aspirate from patients with MDS and associated them with specific clinical features (Bejar et al. 2011). Mutations in TP53, EZH2, ETV6, RUNX1, and ASXL1 were found to be predictors of poor overall survival in MDS patients independently of established risk factors.

## *Personalized Management of Lymphomas*

### Personalized Management B Cell Lymphomas

B cell lymphomas are tumors of cells of the immune system that include Hodgkin's and non-Hodgkin's lymphomas such as follicular lymphoma. B cells are the immune system cells that produce antibodies. Genetic aberrations can cause B cells to

multiply uncontrollably, causing B cell lymphomas. A gene called BCL6 codes for a protein, which is a transcriptional repressor, i.e. it can shut off the functioning of genes in B cells and other cells of the immune system and prevent them from being expressed. The BCL6 protein is normally produced only during a specific stage of B cell development and is never made again. But deregulation of BCL6 can cause the protein to be produced when it should not be. The unwelcome presence of the BCL6 protein blocks the expression of important genes that normally protect cells from becoming cancerous. A peptide called BPI has shown promise in treating B-cell lymphomas by specifically blocking the cancer-causing effects of the BCL6 protein. However, until now, there has been no way to distinguish between diffuse large B cell lymphomas that are caused by BCL6 deregulation and those cases in which BCL6 is expressed but does not actually drive the cancer. In an effort to identify cases of lymphoma that are uniquely susceptible to BPI inhibitor therapy, genomic array ChIP-on-chip was used to identify the cohort of direct BCL6 target genes (Polo et al. 2007). In primary diffuse large B cell lymphomas classified on the basis of gene expression profiles, these BCL6 target genes were clearly differentially regulated in BCR tumors, a subset of DLBCLs with increased BCL6 expression and more frequent BCL6 translocations. Only BCR tumors were highly sensitive to the BCL6 peptide inhibitor, BPI. This genetic signature can help physicians to enroll patients in clinical trials of the new targeted therapy who are most likely to benefit from it. Patients who do not fit this genetic profile will be spared a drug treatment that would be ineffective.

A combination of targeted sequencing and microarray analyses in non-Hodgkin lymphoma cell lines was used in a study to find mutation and gene expression patterns linked to resistance or sensitivity to dacetuzumab, a CD40-stimulating antibody being investigated for the treatment of diffuse large B cell lymphomas and other B cell cancers (Burington et al. 2011). The results showed that a gene expression signature associated with activation of the TNF CD40 pathway can help predict response to a new B cell cancer treatment targeting the pathway. A qRT-PCR assay, which assesses the expression of 15 genes, was subsequently used to predict dacetuzumab treatment response in cell lines, mouse xenograft models, and clinical samples. Generally, cell lines harboring mutations in the tumor suppressor gene p53 were more likely to respond to the CD40 stimulation treatment, as were cell lines with widespread DNA damage or unusually high proliferation rates. Elevated levels of the transcriptional repressor proto-oncogene BCL6 also coincided with treatment sensitivity. In contrast, cell lines exhibiting expression patterns consistent with CD40 pathway activation prior to treatment tended to be resistant. In clinical samples from diffuse large B cell lymphoma patients treated with dacetuzumab during phase I/II trials of the therapy, the response signature accurately predicted treatment response – specifically, tumor shrinkage – for 80 % of the cases. Patients classified as having treatment sensitive tumors based on the expression signature also had significantly longer progression-free survival times following dacetuzumab treatment than those with tumors classified as treatment resistant.

### Personalized Vaccine for Follicular Lymphoma

Follicular lymphoma is considered incurable, although cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy can induce sequential remissions. In one study, patients with follicular lymphoma were vaccinated periodically for more than 2 years with autologous lymphoma-derived idiotype protein vaccine (Inoges et al. 2006). The vaccine presents a tumor protein to the patients in such a way that their immune systems recognize it and destroy any cells bearing that protein. Idiotypic vaccination induced a specific immune response in the majority of patients with follicular lymphoma. Specific immune response was associated with a dramatic and highly statistically significant increase in disease-free survival. This is the first formal demonstration of clinical benefit associated with the use of a human cancer vaccine. Such clinical trials cannot be randomized as each patient serves as his or her own control. A second remission longer than the first would be an indication of efficacy.

### Companion Diagnostic for Treatment of Lymphoma with Adcentris™

Seattle Genetics and Takeda's Millennium are developing Adcetris™ (brentuximab vedotin) jointly. Adcentris (brentuximab vedotin) is an antibody-drug conjugate (ADC), which comprises an anti-CD30 MAb attached by a protease-cleavable linker to a microtubule disrupting agent, monomethyl auristatin E (MMAE), utilizing Seattle Genetics' proprietary technology. The ADC employs a linker system that is designed to be stable in the bloodstream but to release MMAE upon internalization into CD30-expressing tumor cells. Adcentris was granted accelerated approval by FDA in 2011 for treating 2 types of lymphoma: (1) relapsed Hodgkin lymphoma after failure of autologous stem cell transplant or after failure of at least two prior multi-agent chemotherapy regimens; and (2) systemic anaplastic large cell lymphoma after failure of at least one prior multi-agent chemotherapy regimen. It is also in development for a range of other CD30-expressing lymphoma and non-lymphoma malignancies, both as monotherapy and in combination with chemotherapy.

As part of their ongoing clinical development program for the drug, Millennium and Seattle are planning two phase III studies that will incorporate the use of a companion diagnostic, although it is currently not required. One trial will involve patients with CD30-positive cutaneous T-cell lymphoma (CTCL), and the other patients with CD30-positive mature T-cell lymphomas (MTCL). Although the identification of CD30 expression and its role in the diagnosis of Hodgkin lymphoma and systemic ALCL is well established, CD30 expression in other malignancies is more heterogeneous. Under terms of their collaboration Seattle retains US and Canadian commercialization rights to the product, and Takeda has rights to commercialize in the rest of the world. The firms are funding development costs on a 50:50 basis worldwide, except in Japan, where Takeda has sole responsibility for developing Adcentris.

## *Personalized Management of Gastrointestinal Cancer*

### Personalized Management of Esophageal Cancer

Esophageal cancer is highly aggressive malignancy. Almost half of new cases are diagnosed at an advanced stage, when the 5-year survival rate is just 14 %. Surgery is offered to most patients, as well as one or all of the following treatments: an antimetabolite chemotherapy agent (5FU), an alkylating agent (cisplatin) and radiation treatment. Efforts are being made to evaluate esophageal cancer treatment with a pharmacogenetic-based approach that takes into consideration genes in each drug action pathway as a means of developing a more accurate and consistent risk prediction model. Patients with resectable adenocarcinoma or squamous cell carcinoma of the esophagus who have been treated with chemoradiation followed by esophagectomy show that methylenetetrahydrofolate reductase polymorphisms can modify 5-fluorouracil response. This supports the hypothesis that response or resistance to therapy in esophageal cancer patients may be modulated by genetic variants involved in the metabolism or mechanism of chemotherapy drug action. Further research on esophageal cancer aims to determine individual pharmacogenetic profiles to identify patients most likely to have chemotherapeutic benefit and patients with the highest risk of suffering genotoxic side effects. These profiles will ideally lead to individualized therapies, improved treatment outcomes, and a movement toward clinically applied pharmacogenetics. This emergent area of biomedicine could lead to substantially improved clinical outcomes for patients with adenocarcinoma or squamous cell carcinoma of the esophagus. For example, a combination of several gene variants in patients treated with one type of chemotherapy (5-FU) more than doubled survival to in patients treated with the same drug who did not have these variants. The findings represent a significant advance in the goal to provide personalized therapy because it offers a genetic blueprint for gauging the potential effectiveness of all common esophageal cancer treatment, not just an analysis of how one or two “candidate” genes respond to a single treatment. The patients with the best outcomes are those who have gene variants that are less effective at neutralizing the killing power of the cancer treatments. Conversely, patients whose genes efficiently counteract chemotherapy and radiation treatment have shorter survival times overall. If successful, such pathway-based analyses can be conducted for the wide variety of cancers that are treated with 5FU, cisplatin and radiation, as well as other drug treatments.

### Personalized Management of Gastric Cancer

Gastric cancer is the second most common cause of cancer death worldwide with approximately one million cases diagnosed annually. Despite considerable improvements in surgical techniques, innovations in clinical diagnostics and the development of new chemotherapy regimens, the clinical outcome for patients with advanced gastric cancer is generally poor with 5-year survival rates ranging between 5 % and 15 %.

Several molecular therapies are in development for gastric cancer. Cyclooxygenase-2 (COX-2) is overexpressed in and correlated with gastric cancer, and knockdown of COX-2 or administration of COX-2 inhibitors suppresses tumor formation in models of gastric cancer. Induction of apoptosis, reduction of angiogenesis, and blocking of potassium ion channels may present new mechanisms of COX-2 inhibition. Osteopontin is a secreted protein involved in stress response, inflammation, wound healing, and immune response. Inhibition of osteopontin by RNAi technique suppresses tumorigenesis as well as angiogenesis in gastric cancer. Using HLA-A-matched allogeneic gastric cancer cells to induce tumor-specific cytotoxic T lymphocytes is another option for personalized immunotherapy of gastric cancer (Wu et al. 2009).

In ~22 % of cases, gastric cancer is HER2-positive. A phase III study has shown that Herceptin (Genentech/Roche) is effective in advanced HER2-positive stomach cancer and an application to FDA for approval is planned.

**Personalized Management of Colorectal Cancer**

Colorectal cancer (CRC) is one of the most common cancers in the world and is a leading cause of cancer mortality and morbidity. CRC is the second most common cause of cancer death in the US with ~150,000 Americans diagnosed yearly with the disease. The cause of CRC is multifactorial, involving hereditary susceptibility, environmental factors, and somatic genetic changes during tumor progression. Due to a high degree of genetic and pathological heterogeneity, sporadic CRC is considered a collection of diseases that should be approached with different therapeutic strategies. Integration of a new generation of molecularly targeted drugs into the treatment of CRC, coupled with the development of sophisticated technologies for individual tumors as well as patient molecular profiling, form the bases of personalized management of CRC.

Hereditary nonpolyposis CRC (HNPCC) is a familial cancer syndrome characterized by mutations in at least one of six DNA mismatch repair genes: hPMS1, hPMS2, hMSH2, MSH6, hTGFBR2 and hMLH1. From 5 % to 10 % of the 150,000 cases of CRC diagnosed each year in the US are of hereditary type. Identification of DNA microsatellite instability refines the diagnosis of HNPCC, allowing frequent early-onset colonoscopic screening to be restricted to individuals with an especially high risk of this type of cancer. It is possible that a combination of tests for microsatellite instability, allelic loss, p53 mutations, and other genetic alterations in patients with early stage CRC will define groups of patients who require different adjuvant therapies or no systemic treatment at all. Despite the recent results of systemic chemotherapy, more than 40 % of patients with advanced cancer still do not achieve substantial benefits with cytotoxic agents. Therefore, personalized strategies are warranted to improve the probability of disease control. It is important to have a strategy for screening and early detection for preventive measures.

The NCI has developed absolute risk prediction models for CRC from population-based data, and a simple questionnaire suitable for self-administration (Freedman

et al. 2009). The model included a cancer-negative sigmoidoscopy/colonoscopy in the last 10 years, polyp history in the last 10 years, history of CRC in first-degree relatives, aspirin and NSAID use, hormone use, cigarette smoking, body mass index, current leisure-time vigorous activity, and vegetable consumption (www.cancer.gov/colorectalcancerrisk). The absolute risk model for CRC was well calibrated in a large prospective cohort study (Park et al. 2009a). This prediction model, which estimates an individual's risk of CRC given age and risk factors, may be a useful tool for physicians, researchers, and policy makers.

The success of chemotherapy depends on various factors such as gender, age and histological subtype of tumor. The difference in drug effects between different genotypes can be significant. Promising candidates have been identified with predictive value for response and toxicity to chemotherapy in CRC. These candidates need to be incorporated into large, prospective clinical trials to confirm their impact for response and survival to chemotherapy that has been reported in retrospective analyses. Confirmed predictive markers, together with additional yet to be identified pharmacogenomic key players, will provide the basis for tailoring chemotherapy in the future. The rationale for this approach is based on the identification of the in vivo interactions among patient's characteristics, disease physiopathology, and drug pharmacodynamics and pharmacokinetics. Despite the recent encouraging data, the clinical use of targeted therapy is hampered by several questions that need to be answered such as optimal biologic dose and schedule, lack of predictive surrogate biomarkers, and modalities of combination with chemotherapy/radiotherapy. To improve this situation, high throughput methods have been used to discover prognostic and predictive biomarkers for CRC. There is still a need for multiple biomarker testing and to identify panels of predictive biomarkers in order to improve response rates and decrease toxicity with the ultimate aim of tailoring treatment according to an individual patient and tumor profile. Three major genetic and epigenetic alterations that drive CRC tumorigenesis have been identified: microsatellite instability (MSI), chromosomal instability (CIN) and CpG island methylator phenotype (CIMP). These alterations have mainly been used as biomarkers for defining CRC prognosis, but recent data have demonstrated their correlation with treatment response. Utilization of KRAS gene status as a therapeutic biomarker for the administration of EGFR inhibitors is a notable example of how molecular profiling can provide unique advantages for the identification of subpopulations of patients with a high response rate to standard of care.

Most of the targeted inhibitors in development or in clinical use are molecules with high affinity for growth factor receptors, such as FGFR, VEGFR, PDGFR, mast/stem cell growth factor receptor (KITR) and EGFR. Introduction of MAbs that bind to growth factors into the combination chemotherapy regimens currently used in metastatic CRC has been shown to be effective, and has further widened the treatment options. The present scientific consensus is that the large individual differences in treatment response among CRC patients is due to the fact that each patient's tumor is different at the molecular level as a result of the unique genetic and environmental background of that patient (Silvestri et al. 2013). Therefore, an understanding of these molecular differences is essential for optimizing treatment

regimens. For this reason the development and application of individualized therapy has been the goal of several studies within the last decade.

DNA microarray analysis was used to analyze the transcriptional profile of HCT116 CRC cells that were treated with 5-FU or oxaliplatin and selected for resistance to these agents (Boyer et al. 2006). Bioinformatic analyses identified sets of genes that were constitutively dysregulated in drug-resistant cells and transiently altered following acute exposure of parental cells to drug. Functional analysis of three genes identified in the microarray study (prostate-derived factor, calretinin, and spermidine/spermine N1-acetyl transferase) revealed their importance as novel regulators of cytotoxic drug response. These data show the power of this novel microarray-based approach to identify genes which may be important biomarkers of response to treatment and/or targets for CRC.

Panitumumab (Amgen's Vectibix) is a recombinant, human IgG2 kappa MAb that binds specifically to the human EGFR is indicated as a single agent for the treatment of EGFR-expressing, metastatic CRC with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy. A companion diagnostic, TheraScreen K-RAS Mutation Kit (QIAGEN), which was used in the pivotal clinical trial for panitumumab, is available in 22 EU countries. The kit detects seven mutations in codons 12 and 13 of the K-RAS oncogen. Patients with CRC bearing mutated K-RAS do not benefit from cetuximab, whereas patients with a tumor bearing wild-type K-RAS do benefit from cetuximab (Karapetis et al. 2008). The mutation status of the K-RAS gene has no influence on survival among patients treated with best supportive care alone. Launch of this companion diagnostic in 2008 marked the first time that the European Commission licensed a bowel cancer treatment with the stipulation that a predictive test should be carried out. In 2009, the FDA updated the "indication and usage" section of the labels of Vectibix and Erbitux (ImClone/Bristol-Myers Squibb) to note that retrospective analyses of metastatic CRC have not shown a treatment benefit for the EGFR inhibitors in patients whose tumors had KRAS mutations in codon 12 or 13, and that the use of the drugs is not recommended for the treatment of CRC patients with these mutations. Physicians can now eliminate anti-EGFR antibodies as a treatment option for patients with mutated KRAS tumors and redirect those patients to alternative therapies, avoiding unnecessary treatments in patients who are unlikely to benefit. Other activating RAS mutations may also be negative predictive biomarkers for anti-EGFR therapy in patients with metastatic CRC. In patients who had metastatic CRC without RAS mutations, improvements in overall survival have been observed with panitumumab plus oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) therapy (Douillard et al. 2013).

In general, CRC prognosis is based on clinical staging, with roughly 40 % of cases diagnosed in early or localized stages. Patients with stage I and II colon cancer are often considered cured following surgery. Nevertheless, some 15–20 % of these individuals eventually have recurrence of the disease. Therefore, efforts are being made to define the molecular changes associated with recurrence and decreased survival. Since high tumor grade is not a biomarker of higher recurrence risk in stage II colon cancer, suggests that other biomarkers, such as Genomic Health's

Oncotype DX® Recurrence Score® test as well as T-stage and mismatch repair status, should be considered during the treatment decision-making process.

Interest is focused on DNA methylation, an epigenetic mechanism that is involved in everything from imprinting to X-chromosome inactivation, for determining prognosis of CRC. The results of an analysis of the methylation patterns using pyrosequencing in CRC samples taken from two independent prospective cohorts suggest that decreased methylation in regions of the genome called long interspersed nucleotide element-1 (LINE-1) elements is independently associated with poor survival outcomes (Ogino et al. 2008). A 30 % decrease in LINE-1 methylation doubled the risk of CRC-specific mortality, and the lower the methylation level, the worse the patient outcomes. Methylation changes associated with mortality may reflect genomic instability, transcriptional dysregulation, and the activation of oncogenes, inflammation, or oxidative stress. Although follow-up studies are still needed, there are good prospects of clinical application of the results.

Another study has identified a 50-gene signature in early-stage colon cancer that predicts cancer recurrence (Garman et al. 2008). The investigators compiled gene expression data from publicly available datasets, assessing the expression patterns in 52 samples taken from individuals with known survival outcomes. This signature included RAS and TNF family genes previously implicated in carcinogenesis as well as genes in several pathways linked to metastasis. The team validated nine of the top 10 differentially expressed genes using RT-PCR. Along with its prognostic implications, preliminary results suggest that the signature, which was validated in two independent patient groups, may also provide clues for treating colon cancer. Examination of gene expression in early-stage CRC revealed certain patterns that seem to put some patients at higher risk for recurrence. The signature could detect recurrence with more than 90 % accuracy regardless of the early colon cancer's tumor, node, metastasis, or TNM stage. Identification of these patients may enable targeted and proactive treatment to prevent this recurrence. The investigators also tested whether the gene signature was useful for guiding individuals' treatment and identifying new drugs. Using the Broad Institute's Connectivity Map, they assessed the gene expression profiles of cells treated with a range of drugs to look for profiles resembling the cancer recurrence signature. Their research suggests that at least four drugs may influence the genes involved in the recurrence signature. Subsequent experiments indicated that cell lines with the high recurrence risk signature are sensitive to at least two of these compounds: the COX2 inhibitor celecoxib and the PI3K inhibitor LY-294002. That, in turn, suggests it may be useful to test the treatments in those with the high-risk signature in order to identify patients who may benefit from such treatments rather than standard chemotherapy. This will individualize the treatment plans for patients with CRC and improve survival. Clinical trials are planned to test this.

Identification of genetic factors underlying drug response in CRC still remains a promising area for improving management of CRC patients. Genetic variations identified in genes encoding thymidylate synthase, dihydropyrimidine dehydrogenase, glutathione S-transferase pi, and uridine diphosphate glucosyltransferase 1A1 seem to be promising predictors of drug efficacy and/or toxicity in CRC (Fogli and

Caraglia 2009). Additional investigation is needed to validate the clinical relevance of individual genetic differences.

In 2011, OncoTrack, an international consortium of academic researchers, pharmaceutical companies, and commercial partners, launched a 5-year project to develop and assess new biomarkers for CRC. Total budget for the project along with funding from the Innovative Medicines Initiative – a private-public partnership between the pharmaceutical industry and the European Union – amounts to €25.8 million ($35.6 million). OncoTrack was founded to create next-generation methods of biomarker development to develop personalized treatment of CRC. The consortium, led by Bayer HealthCare Pharmaceuticals and the Max Planck Institute for Molecular Genetics in Germany, includes AstraZeneca, Boehringer Ingelheim, Janssen Pharmaceutica, Merck, Pfizer, and Roche Diagnostics. Academic partners include Uppsala University, University College London, Paris South University, Charité Universitätsmedizin Berlin, Medizinische Universität Graz, and Technische Universität Dresden. International Prevention Research Institute, Experimental Pharmacology and Oncology, and Alacris Theranostics also are members of OncoTrack. The consortium's first project called "Methods for systematic next-generation oncology biomarker development" will seek to generate high-quality genomic and epigenetic data from clinically well-defined CRC tumors and their metastases. The data will be compared to the germline genome of the patients, and will be complemented by a detailed molecular characterization of the tumors. OncoTrack will establish and characterize a new series of xenograft tumor models and cell lines derived from the same set of tumors in order to support tumor biology research and the early stages of biomarker qualification. The combined data from all phases of the project will enable OncoTrack to address fundamental questions regarding the relationship between tumor genotype and phenotype, thus providing the starting point for discovery and selection of suitable candidates for development as biomarkers of CRC.

### Sequencing for Personalized Management of Colorectal Cancer

The Cancer Genome Atlas project plans to profile genomic changes in 20 different cancer types and has now presented results from multidimensional analyses of human CRC. The distinction between the colon and the rectum is largely anatomical, but it has both surgical and radiotherapeutic management implications as well as an impact on prognosis. Most investigators divide CRC biologically into those with microsatellite instability (MSI; located primarily in the right colon and frequently associated with the CpG island methylator phenotype and hyper-mutation) and those that are microsatellite stable but chromosomally unstable.

Previous investigations have uncovered several critical genes and pathways that are important in the initiation and progression of CRC. These include the WNT, RAS-MAPK, PI3K, TGF-β, P53 and DNA mismatch-repair pathways. Large-scale sequencing analyses have identified numerous recurrently mutated genes and a recurrent chromosomal translocation, but a fully integrated view of the genetic and

genomic changes and their significance for CRC tumorigenesis was lacking. Genomic patterns that have now been uncovered in CRC, including samples originating at sites in either the colon or the rectum, reveal genomic profiles that are similar to those present in tumors at each site (The Cancer Genome Atlas Network 2012). By doing sequencing, CNV analyses, and/or methylation profiling on almost 300 CRC samples, the team narrowed in on key genes and pathways that tend to be altered in CRC. For the new analysis, researchers used SOLiD or Illumina sequencing platforms to sequence the exomes of 224 tumor-normal pairs to an average depth of >20 X over 80 % or more of the coding sequences targeted. With the Illumina HiSeq 2000, they also did low coverage WGS on 97 of the tumors and matched normal samples. The transcriptional analysis was expanded further through RNA sequencing and miRNA sequencing experiments. The data presented provide a useful resource for understanding CRC and identifying possibilities for treating it in a targeted way. Although it may take years to translate this foundational genetic data on CRC into new therapeutic strategies and surveillance methods, this genetic information will be the springboard for determining what will be clinically effective against CRC. A subset of the CRC, most often tumors showing up in the right or ascending colon, had unusually high mutation levels. Approximately 16 % of the tumors could be classified as hypermutated, containing a median of 728 predicted somatic mutations apiece.

More than 75 % of these hypermutated samples showed enhanced methylation levels and microsatellite instability. As the survival rate of patients with high microsatellite instability-related cancers is better and these cancers are hypermutated, mutation rate may be a better prognostic indicator.

From their genome sequence data, researchers tracked down several suspected translocation events involving bits of sequence from different chromosomes. For example, 3 of the 97 tumors assessed by low-coverage genome sequencing contained a fusion linking the first exons of the chromosome 11 gene NAV2 to part of the chromosome 2 gene TCF7L1, which codes for a component in the WNT pathway, a known contributor to CRC. Almost all of the tumors from both the hypermutated and the non-hypermutated groups included mutations expected to boost the activity of the WNT signaling pathway and to curb signaling via the TGF-β pathway, changes that are expected to produce an increase in the activity of the MYC proto-oncogene. These findings fit with early reports suggesting that compounds targeting that pathway may be effective against some CRCs. Possible therapeutic approaches to CRC included WNT-signaling inhibitors and small-molecule β-catenin inhibitors, which are showing initial promise. Other commonly affected pathways included the RTK-RAS, MAP kinase, TP53, and PI3 kinase pathways, which point to potential targets for new CRC treatments. Approximately 5 % of the CRC tumors studied had extra copies of a gene, ERBB2, as do many breast cancer tumors. The drug, Herceptin, which is effective for breast cancer patients with too many ERBB2 genes, might also help CRC patients with the same aberration. Clinical trials have been proposed to test the effects of Herceptin in these CRC patients. Approximately 15 % of CRCs had a mutation in a gene, BRAF that is also often mutated in melanoma. A drug approved for melanoma blocks the function of BRAF gene product, but it has not worked in CRC patients.

### Systems Biology Approach to Drug Resistance in Colorectal Cancer

Mechanisms that may have important implications for drug efficacy and actively contribute to innate resistance in colorectal cancer (CRC) are:

- High levels of thymidylate synthase, the 5-FU target, are associated with tumor insensitivity to FU-based therapy.
- Higher levels of topoisomerase-I (TOP1) correlate with greater sensitivity of colon tumors to camptothecin derivatives compared to normal colonic mucosa.
- Glucuronidation, involved in xenobiotic detoxification, is also associated with innate resistance to TOP1 inhibitors in colon cell lines and tumors.
- An increase of the ABCB1/P-gp transporter, a member of the family of ABC-transporters that detect and eject anticancer drugs from cells, is observed in intrinsically drug-resistant colon tumors.

In a systems biology approach to understand innate CRC tumor responses to a FOLFIRI combined chemotherapy of irinotecan (CPT-11) plus 5-FU/FA, gene expression patterns obtained with microarrays were compared between clinical samples from colon tumors and liver metastases collected from CRC patients prior to drug exposure (Grauden et al. 2006). Use of a vigilant experimental design, power simulations and robust statistical analysis reduced the false negative and positive differential hybridization rates to a minimum. Data collected from a biological systems perspective into global and interconnected molecular networks highlight the molecular mechanisms that may anticipate resistance in CRC patients prior to their exposure to drugs. This knowledge could be used in clinical practice as a complement to clinical, biochemical and genetic biomarkers for global prevention, early diagnosis and better patient treatment.

### Resistance to Targeted EGFR Blockade in CRC

CRC that is wild type for KRAS is often sensitive to EGFR blockade, but almost always develop resistance within several months of initiating therapy. This situation is in marked contrast to that of small-molecule targeted agents, such as inhibitors of ABL, EGFR, BRAF and MEK, in which mutations in the genes encoding the protein targets render the tumors resistant to the effects of the drugs. Two studies have provided an explanation of why solid tumors develop resistance to targeted therapies in a highly reproducible fashion and provide a basis for overcoming this. One study of circulating tumor DNA found that 38 % patients whose tumors were initially KRAS wild type developed detectable mutations in KRAS in their sera and some of them developed multiple different KRAS mutations (Diaz et al. 2012). The appearance of these mutations was very consistent, generally occurring between 5 and 6 months following treatment. Mathematical modeling indicated that the mutations were present in expanded subclones before the initiation of panitumumab treatment. These results suggest that the emergence of KRAS mutations is a

mediator of acquired resistance to EGFR blockade and that these mutations can be detected in a noninvasive manner.

A second study found that cetuximab, a MAb that binds the extracellular domain of EGFR, is effective in a subset of KRAS wild-type metastatic CRC, but after an initial response, secondary resistance invariably ensues, thereby limiting the clinical benefit of this drug. Further investigations showed that point mutations of KRAS are causally associated with the onset of acquired resistance to anti-EGFR treatment in CRC, but resistant cells remained sensitive to combined inhibition of EGFR and mitogen-activated protein-kinase kinase (Misale et al. 2012). Analysis of metastases from patients who developed resistance to cetuximab or panitumumab showed the emergence of KRAS amplification in one sample and acquisition of secondary KRAS mutations in 60 % of the cases. KRAS mutant alleles were detectable in the blood of cetuximab-treated patients as early as 10 months before radiographic documentation of disease progression. In summary, the results identify KRAS mutations as frequent drivers of acquired resistance to cetuximab in CRC, indicate that the emergence of KRAS mutant clones can be detected non-invasively months before radiographic progression and suggest early initiation of a MEK inhibitor as a rational strategy for delaying or reversing drug resistance.

## *Personalized Management of Liver Cancer*

Liver cancer is the second-leading cause of cancer-related deaths worldwide, killing >600,000 people annually. If hepatocellular carcinoma (HCC), the most common type of liver cancer, is diagnosed in its early stages, it can be treated by surgically removing part of the liver, by liver transplantation, or by local ablation using an electric current to destroy the cancer cells. HCC occurs mainly in men.

Astrocyte elevated gene-1 (AEG-1) is overexpressed in >90 % of human hepatocellular carcinoma (HCC) patients and plays a significant role in mediating aggressive progression of HCC. AEG-1 is known to augment invasion, metastasis, and angiogenesis, and now has been shown to directly contributes to another important hallmark of aggressive cancers, that is, resistance to chemotherapeutic drugs, such as 5-FU (Yoo et al. 2009). AEG-1 augments expression of the transcription factor LSF that regulates the expression of thymidylate synthase, a target of 5-FU. In addition, AEG-1 enhances the expression of dihydropyrimidine dehydrogenase (DPYD) that catalyzes the initial and rate-limiting step in the catabolism of 5-FU. siRNA-mediated inhibition of AEG-1, LSF, or DPYD significantly increases the sensitivity of HCC cells to 5-FU in vitro and a lentivirus delivering AEG-1 siRNA in combination with 5-FU markedly inhibited growth of HCC cells xenotransplanted in athymic nude mice when compared to either agent alone. Thus AEG-1 and LSF genes contribute to chemoresistance. Inhibition of AEG-1 can be exploited as a therapeutic strategy along with 5-FU-based combinatorial chemotherapy for HCC.

IntegraGen is developing a panel of biomarkers, which have been shown to be prognostic for outcomes in patients with hepatocelluar carcinoma (liver cancer). By studying the expression of genes, IntegraGen aims to better predict survival

outcomes and the potential for cancer recurrence in patients with HCC and to identify differing subgroups of patients where surgery and targeted therapies are more effective. Pfizer is evaluating IntegraGen's 56-gene molecular signature of HCC for classification into six categories to predict prognosis.

Interferon (IFN) has a significant beneficial effect after curative treatment of HCC in terms of both survival and tumor recurrence. A multikinase inhibitor sorafenib has also been reported to enhance survival. Despite several treatment options, fewer than half of candidates for potentially curative treatments receive them.

Targeted adjuvant therapies require methods to guide selection of optimal treatment for HCC. HCC can be classified in molecular classes according to Wnt-beta-catenin pathway activation, proliferation signature activation (associated with chromosomal instability), and other subgroups. A molecular classification is essential to enable the development of new targets, and to customize therapies in patients with HCC (Villanueva et al. 2008).

The expression patterns of miRNAs in liver tissue differ between men and women with hepatocellular carcinoma. The miR-26 expression status of such patients is associated with survival and response to adjuvant therapy with IFN-α. A study showed that patients whose tumors had low miR-26 expression had shorter overall survival but a better response to IFN-α than patients whose tumors had high expression of the miRNA (Ji et al. 2009). This has implications for the personalized management of liver cancer.

#### Prediction of Recurrence of Hepatocellular Carcinoma

Typically observed at 2 years after surgical resection, late recurrence is a major challenge in the management of HCC. Systematic analysis of gene expression data from human liver undergoing hepatic injury and regeneration revealed 233-gene signature that was significantly associated with late recurrence of HCC (Kim et al. 2014b). Using this signature, the authors developed a prognostic predictor that can identify patients at high risk of late recurrence validated the robustness of the predictor in patients. The potential significance of STAT3 activation in late recurrence was predicted by gene network analysis and validated later. Two independently developed predictors reflected well the differences between early and late recurrence of HCC at the molecular level and provided new biomarkers for risk stratification. The main limitation of the study is that most of the patients were hepatitis B virus-positive. Further investigations are needed to test these prediction models in patients with different causes of HCC, such as hepatitis C virus.

### *Personalized Management of Lung Cancer*

Globally, >1.6 million new cases of lung malignancies are diagnosed annually with about 85 % having NSCLC, who often have advanced disease and a low survival rate. It is important to have targeted therapies as well as companion diagnostics to guide the selection of patients most likely to respond to these treatments.

### Crizotinib for Personalized Management of NSCLC

Crizotinib (Pfizer's Xalkori) is an orally available small molecule that blocks fusion proteins of anaplastic lymphoma kinase (ALK). This fusion, EML4 (echinoderm microtubule associated protein like 4)-ALK is a tyrosine kinase like BCR-ABL, and its formation drives tumor formation; therefore, blocking it should halt tumor growth. In a study of 1,500 patients with NSCLC, 5.5 % had a fusion containing ALK, and 57 % of them responded well to crizotinib (Kwak et al. 2010). The FDA has cleared for marketing Abbott's companion diagnostic kit, Vysis ALK Break Apart FISH Probe, for use with crizotinib to detect rearrangements of the anaplastic lymphoma kinase gene in NSCLC. The kit uses FISH technology to detect rearrangements on the ALK gene on the 2p23 chromosome, providing clinicians a standardized, clinically validated method to identify those patients who may benefit the most from Pfizer's drug. The test is designed to identify the 3–5 % of all NSCLC patients who would be candidates for Xalkori, and this is expected to change how patients with NSCLC are diagnosed and treated.

Despite these remarkable initial responses, cancers eventually develop resistance to crizotinib, usually within 1 year, thereby limiting the potential clinical benefit. A study enumerates the mutations in EML4-ALK that confer resistance to crizotinib (Choi et al. 2010). Using a model of acquired resistance to ALK inhibitors, it was shown that second-generation ALK TKIs or Hsp90 inhibitors are effective in treating crizotinib-resistant tumors harboring secondary gatekeeper mutations (Katayama et al. 2011).

### Ceritinib

Ceritinib (LDK378, Novartis) is a new ALK inhibitor that has shown greater antitumor potency than crizotinib in preclinical studies. NSCLC harboring ALK gene rearrangement is sensitive to the ALK inhibitor crizotinib, but resistance invariably develops. In a phase I clinical trial, ceritinib was highly active in patients with advanced, ALK-rearranged NSCLC, including those who had had disease progression during crizotinib treatment, regardless of the presence of resistance mutations in ALK (Shaw et al. 2014). Responses were observed in patients with various resistance mutations in ALK and in patients without detectable mutations. Among patients with NSCLC who received at least 400 mg of ceritinib per day, the median progression-free survival was 7 months.

### EGFR Tyrosine Kinase Inhibitor Treatment

The tyrosine kinase inhibitor (TKI) gefitinib (Iressa), which targets the epidermal growth factor receptor (EGFR), is approved for late cases of non-small-cell lung cancer (NSCLC) as a last resort treatment. Most of NSCLC patients do not respond to gefitinib but about 10 % of patients have a rapid and often dramatic clinical

response. The molecular mechanisms underlying sensitivity to gefitinib are unknown. It was considered to be a targeted therapy based on the idea that lung cancer might produce excess of EGFR, and blocking it might slow growth with less toxicity than standard chemotherapy. This growth protein contains a little pocket to capture ATP. Gefitinib apparently targets that pocket, and when the protein is mutated, gefitinib fits inside the pocket much better, blocking ATP and thus inhibiting cancer-cell growth. Patients with lung cancer who respond to gefitinib have been reported to have somatic mutations consisting of deletions in exon 19 and in exon 21 of the EGFR gene. In addition, a mutation in exon 20 is also associated with acquired resistance to gefitinib in initially gefitinib-sensitive patients.

Laboratory studies of cancer cells show that the mutated receptors are 10 times more sensitive to gefitinib than normal receptors. The mutations are more common in women, nonsmokers, and persons who had a subtype called bronchoalveolar cancer. EGFR mutations have been reported in lung cancer samples from patients who responded to gefitinib (Eli Lilly & Co's Iressa) therapy and in a lung adenocarcinoma cell line that was hypersensitive to growth inhibition by gefitinib, but not in gefitinib-insensitive tumors or cell lines. These results suggest that EGFR mutations may predict sensitivity to gefitinib. Increased EGFR gene copy number based on FISH analysis is a good predictive biomarker for response to EGFR inhibitors, stable disease, time to progression, and survival in NSCLC. However, EGFR mutation is a better predictor of clinical outcome in gefitinib-treated patients than the CNV's of EGFR gene. These findings are important as they would enable the development of personalized treatment of cancer. The EGFR Mutation Assay (Genzyme) detects EGFR mutations in patients with NSCLC that correlate with clinical response to Tarceva® (erlotinib) and Iressa® (gefitinib). This would enable treatment of responders and even at an earlier stage than the current practice of using it as a last resort. Prospective large scale clinical studies must identify the most optimal paradigm for selection of patients.

Ficlatuzumab (Aveo Oncology) is a HGF inhibitory antibody in an exploratory phase II study as first-line treatment of NSCLC patients and VeriStrat (Biodesix), a commercially available test that helps physicians guide treatment decisions for NSCLC patients, is being developed as a companion diagnostic under an agreement between the two firms. VeriStrat can gauge prognosis of patients treated with EGFR inhibitors that are also TKIs and predict which patients would have better progression-free survival from ficlatuzumab plus a TKI rather than from a TKI alone. A proof-of-concept trial of ficlatuzumab plus erlotinib (Pfizer's Tarceva) will be conducted in advanced NSCLC. Patients in this study will be selected using VeriStrat.

Many patients with NSCLC who show radiographic responses to treatment with EGFR tyrosine kinase inhibitors gefitinib and erlotinib have somatic mutations in the EGFR tyrosine kinase domain. Both are known as small-molecule drugs that can be taken orally and block the part of the EGFR molecule that's located within the cell. Combined use of gefitinib and cetuximab (Erbitux), a MAb for CRC, shows that although both drugs kill cells containing a normal but overactive EGFR molecule, only gefitinib kills lung cancer cells containing a mutated EGFR molecule

whereas cetuximab has little effect on the mutant signal, evidently because it strikes at a different part of the EGFR molecule. Thus those with EGFR mutations benefit from gefitinib or erlotinib, whereas those without EGFR mutations benefit from cetuximab. Cetuximab binds to a portion of the EGFR receptor that extends outside the cell. This difference in action is the apparent explanation for why they perform differently against the mutant EGFR cells. These findings show that in order to inhibit the mutant receptor, one should inhibit the domain of the EGFR molecule that lies within the cell, as opposed to the extracellular domain.

Previously, tumor biopsies have been used in NSCLC for EGFR genotyping as it has been difficult to detect the low levels of specific mutations shed from the tumor into the blood against the high background of normal DNA. Testing DNA isolated from blood, rather than tumor tissue, would be better for predicting responses to gefitinib, erlotinib (Tarceva) and other cancer therapies. If EGFR mutations can be observed in serum DNA, this could serve as a noninvasive source of information on the genotype of the original tumor cells as compared to direct sampling of the tumor and could influence treatment and the ability to predict patient response to gefitinib. In one study, serum genomic DNA was obtained from Japanese patients with NSCLC before first-line gefitinib monotherapy (Kimura et al. 2006). Scorpion Amplified Refractory Mutation System technology (QIAGEN) was used to detect EGFR mutations. In pairs of tumor and serum samples obtained from patients, the EGFR mutation status in the tumors was consistent with those in the serum of >72 % of the paired samples. The QIAGEN test kit detected mutations that were missed by direct sequencing techniques. These results suggest that patients with EGFR mutations seem to have better outcomes with gefitinib treatment, in terms of progression-free survival, overall survival, and response, than those patients without EGFR mutations. TheraScreen EGFR 29 Mutation Test (QIAGEN) detects mutations that correlate with responsiveness to EGFR tyrosine kinase inhibitors. Therascreen EGFR RGQ Plasma PCR was CE-marked in Europe in January 2015 for marketing in >30 European countries, making it first-ever regulated companion diagnostic assay that has demonstrated clinical utility for guiding treatment decisions in patients with solid tumors based on the analysis of molecular biomarkers obtained from a body fluid (liquid biopsy). This test may be used to help physicians choose NSCLC patients who are most likely to respond to treatment with EGFR tyrosine kinase inhibitors. European Medicines Agency has extended the drug label of Iressa® to include the detection of EGFR mutations in circulating tumor DNA obtained from a blood sample when a tumor sample is not evaluable.

Dacomitinib (Pfizer) is an oral, once-daily, pan-HER inhibitor. It is an irreversible inhibitor of HER-1 (EGFR), HER-2 and HER-4 tyrosine kinases. Dacomitinib targets multiple receptors of the HER pathway, whereas currently marketed HER-1 (EGFR) inhibitors for NSCLC target only one receptor in this pathway. QIAGEN is developing a companion diagnostic for dacomitinib be based on its proprietary KRAS assay technology, which reliably detects mutations of the KRAS gene that are frequently found in human cancers. Because EGFR inhibitors are generally effective in patients without these KRAS mutations, the QIAGEN assay can be useful in identifying patients most appropriate for EGFR-inhibitor therapies.

In another approach to this problem, serum collected from NSCLC patients before treatment with gefitinib or erlotinib were analyzed by MALDI MS and spectra were acquired independently at two institutions (Taguchi et al. 2007). An algorithm to predict outcomes after treatment with EGFR tyrosine kinase inhibitors was developed from a training set of patients from three cohorts. The algorithm was then tested in two independent validation cohorts of patients who were treated with gefitinib and erlotinib and in three control cohorts of patients who were not treated with EGFR tyrosine kinase inhibitors. The clinical outcomes of survival and time to progression were analyzed. This MALDI MS algorithm was not merely prognostic but could classify NSCLC patients for good or poor outcomes after treatment with EGFR tyrosine kinase inhibitors. This algorithm may thus assist in the pretreatment selection of appropriate subgroups of NSCLC patients for treatment with EGFR tyrosine kinase inhibitors. The test is commercially in development by Biodesix Inc.

One study involving EGFR mutational analysis on DNA recovered by CTC-Chip from circulating tumor cells using allele-specific PCR amplification has compared the results with those from concurrently isolated free plasma DNA and from the original tumor-biopsy specimens (Maheswaran et al. 2008). Thus molecular analysis of circulating tumor cells from the blood of patients with lung cancer offers the possibility of monitoring changes in epithelial tumor genotypes during the course of treatment.

A study from Spain has evaluated the feasibility of large-scale screening for EGFR mutations in advanced NSCLC and analyzed the association between the mutations and the outcome of erlotinib treatment (Rosell et al. 2009). It concluded that large-scale screening for EGFR mutations is feasible with subsequent customization of erlotinib and improved outcome. It is warranted in women with lung cancer, in those who have never smoked, and in those with nonsquamous tumors.

### Development of Resistance to EGFR Inhibitors

Acquired resistance to EGFR TKIs is inevitable in metastatic EGFR-mutant lung cancers. Because RAS/RAF/MEK mutations are known mediators of acquired resistance in other solid tumors (colon cancers, gastrointestinal stromal tumors, and melanomas) responsive to targeted therapies, the frequency of secondary KRAS/NRAS/BRAF/MEK1 gene mutations was analyzed in the largest collection to date of lung cancers with acquired resistance to EGFR TKIs (Ohashi et al. 2012). No recurrent NRAS, KRAS, or MEK1 mutations were found in most of patient samples but only 1 % were found to have mutations in BRAF. Ectopic expression of mutant NRAS or BRAF in drug-sensitive EGFR-mutant cells conferred resistance to EGFR TKIs that was overcome by addition of a MEK inhibitor. Collectively, these positive and negative results provide deeper insight into mechanisms of acquired resistance to EGFR TKIs in lung cancer and should be taken into consideration in ongoing clinical trials designed to overcome resistance. In the context of emerging knowledge about mechanisms of acquired resistance to targeted therapies in various cancers, these data highlight that, even though solid tumors share common signaling

cascades, mediators of acquired resistance must be elucidated for each disease separately in the context of treatment.

Patients with EGFRm + NSCLC are particularly sensitive to treatment with currently available EGFR TKIs, which block the cell signaling pathways that drive the growth of tumor cells. However, tumor cells almost always develop resistance to treatment, leading to disease progression. In approximately half of patients, this resistance is caused by the secondary mutation known as T790M. AZD9291 (AstraZeneca) is a highly selective, irreversible inhibitor of both the activating sensitizing EGFR mutation (EGFRm+) and the activating resistance mutation, T790M, while sparing the activity of wild type EGFR. There are currently no targeted therapies approved for the treatment of tumors with this resistance mutation. In the ongoing phase I study, AZD9291 has shown early evidence of activity as a once-daily monotherapy with clinical responses observed in an EGFRm + population of patients with NSCLC who have previously failed on EGFR TKIs and also in patients with the T790M mutation. AZD9291 has been well-tolerated with low rates of side effects. AstraZeneca is collaborating with Roche to develop a plasma-based companion diagnostic test for EGFR mutations in both tumor tissue and plasma derived from patients with NSCLC, and to optimize the clinical development of AZD9291.

**Molecular Subtyping of Lung Cancer**

Lung adenocarcinoma (LAD) has extreme genetic variation among patients, which is not well understood and limits progress in research and development of therapy. LAD molecular subtypes are a validated stratification of naturally-occurring gene expression patterns and encompass different functional pathways and patient outcomes. Different subtypes may be the result of mutations and alterations in gene expression. LAD molecular subtypes (bronchioid, magnoid, and squamoid) were tested for association with gene mutations and CNVs using statistical methods and published cohorts (Wilkerson et al. 2012). A novel validation cohort was assayed and interrogated to confirm subtype-alteration associations. Mutation rates of genes (EGFR, KRAS, STK11, and TP53), chromosomal instability, regional copy number, and genome wide DNA methylation were significantly different among tumors of the molecular subtypes. Secondary analyses compared subtypes by integrated alterations and patient outcomes. Tumors having integrated alterations in the same gene associated with the subtypes, e.g. mutation, deletion and underexpression of STK11 with magnoid, and mutation, amplification, and overexpression of EGFR with bronchioid. The subtypes also associated with tumors having concurrent mutant genes, such as KRAS-STK11 with Magnoid. Overall survival of patients, cisplatin plus vinorelbine therapy response, and predicted gefitinib sensitivity were significantly different among the subtypes. The study concluded that LAD intrinsic molecular subtypes co-occur with grossly distinct genomic alterations that affect response to therapy. These results advance the understanding of etiology of LAD and help in selection of patient subgroups for future evaluation of treatment response. Lung Subtype Platform (LSP™) is being developed commercially by GeneCentric.

### Personalized Therapy of NSCLC Based on KIF5B/RET Fusion Oncogene

Although several studies have reported genomic driver mutations in NSCLC over the past decade, the molecular pathogenesis of more than 40 % of NSCLC is still unknown. To identify new molecular targets in NSCLC, the combined analysis of massively parallel whole-genome and transcriptome sequencing for cancer was performed on an adenocarcinoma patient, who is a nonsmoker and has no family history of cancer (Ju et al. 2012). The cancer showed no known driver mutation in EGFR or KRAS and no EML4-ALK fusion. However, a novel fusion gene between KIF5B and RET proto-oncogene was found, which is caused by a pericentric inversion of 10p11.22-q11.21. This fusion gene overexpresses chimeric RET receptor tyrosine kinase, which could spontaneously induce cellular transformation. KIF5B-RET fusion has been identified in a few more cases indicating that a subset of NSCLC could be caused by a fusion of KIF5B and RET, and suggest the chimeric oncogene as a promising molecular target for the personalized diagnosis and treatment of lung cancer.

### Predicting Response of NSCLC to Platinum-Based Therapy

Platinum-based chemotherapy is a primary treatment for patients with advanced NSCLC. There is need for a convenient method is to identify the sensitivity of individual patient to platinum-based regimen. Genetic variants in DNA repair genes represent important determinants of drug efficacy. Xeroderma pigmentosum group A (XPA) codon23 and xeroderma pigmentosum group D (XPD) codon751 SNPs are involved in clinical response to platinum-based chemotherapy in advanced NSCLC patients. A study has confirmed that XPA A23G, a SNP in blood cells detected by 3D polyacrylamide gel-based DNA microarray method, might be a promising biomarker in predicting favorable prognosis of NSCLC patients and designing individualized treatments (Cheng et al. 2013).

### Proteomics for Discovery of Metabolic Biomarkers of Lung Cancer

Human primary lung adenocarcinoma tumors have been analyzed using global MS to elucidate the biological mechanisms behind relapse after surgery (Pernemalm et al. 2013). In total, >3,000 proteins were identified with high confidence and supervised multivariate analysis was used to select 132 proteins separating the prognostic groups. Based on in-depth bioinformatics analysis, the authors hypothesized that the tumors with poor prognosis had a higher glycolytic activity and HIF activation. By measuring the bioenergetic cellular index of the tumors, they could detect a higher dependency of glycolysis among the tumors with poor prognosis. Further, they could also detect an up-regulation of HIF1α mRNA expression in tumors with early relapse. Finally, they selected three proteins that were upregulated in the poor

prognosis group (cathepsin D, ENO1, and VDAC1) to confirm that the proteins indeed originated from the tumor and not from a stromal or inflammatory component. Overall, these findings show how in-depth analysis of clinical material can lead to an increased understanding of the molecular mechanisms underlying tumor progression. This study shows a functional coupling between high glycolytic activity and postsurgical relapse of adenocarcinoma of the lung. Protein level changes detected in this study could serve as starting point for discovery of predictive biomarkers for metabolic treatment options in lung cancer.

### Role of microRNAs as Biomarkers of Lung Cancer

Alterations in expression profiles of microRNAs (miRNAs) are linked to lung cancer. Serum miR-148a, miR-148b, and miR-152 are significantly downregulated in NSCLC patients, whereas there is overexpression of serum miR-21 (Yang et al. 2014). The combination of these four candidate miRNAs exhibits higher predictive accuracy in NSCLC screening compared with individual miRNAs. Rule discovery followed by distance separation is a powerful computational method to identify reliable miRNA biomarkers (Song et al. 2014). For example, if the expression level of miR-98 is >7.356 and the expression level of miR-205 is <9.601, the sample is normal rather than cancerous with specificity and sensitivity both 100 %.

miRNAs have been shown to control the expression of cognate target genes and predict relapse in surgically resected NSCLC patients. Overexpression of the Wingless-type (Wnt) genes and methylation of Wnt antagonists have been documented in NSCLC. Understanding the relevance of these findings can help to change the clinical practice in oncology towards customizing chemotherapy and targeted therapies, leading to improvement in both survival and in cost-effectiveness.

### Role of a New Classification System in the Management of Lung Cancer

Apart from genotyping, a new staging system that was developed by the International Association for the Study of Lung Cancer will have a considerable impact on the future management of lung cancer. Changes in the new classification include: creating more sub-stages for tumor size, reassigning some large tumors to a more advanced stage, reclassifying tumors that have spread into the fluid surrounding the lung, and recognizing that spread to certain lymph nodes is more dangerous than its spread to others. By changing these groupings, some patients will get moved to an earlier stage of disease that may be treated more aggressively. For example, a patient may have only been offered chemotherapy but may now be offered chemotherapy and radiation or more intense radiation. Conversely, some people considered to have earlier-stage tumors now will be grouped with those whose tumors have widely spread and discouraged from undergoing therapies that have little chance of helping them.

### Selecting Therapy of Cancer Arising from Respiratory Papillomatosis

In a case of recurrent respiratory papillomatosis with progressive, bilateral tumor invasion of the lung parenchyma, conditional reprogramming was used to generate cell cultures from the patient's normal and tumorous lung tissue. Analysis revealed that the laryngeal tumor cells contained a wild-type 7.9-kb human papillomavirus virus type 11 (HPV-11) genome, whereas the pulmonary tumor cells contained a 10.4-kb genome (Yuan et al. 2012). The increased size of the latter viral genome was due to duplication of the promoter and oncogene regions. The spread of the tumor in the lung was most likely due to the distal aspiration of tumor cells rather than reinfection of new cells. Finally, the finding that the laryngeal tumor lacked the 10.4-kb genome suggests that duplication in the viral genome did not precede extension into the lung. Chemosensitivity testing identified vorinostat as a potential therapeutic agent, which led to stabilization of tumor size with durable effects. This is a good example of use of biotechnology to understand the spread of tumor in an individual patient and selection of appropriate therapy.

### Testing for Response to Chemotherapy in Lung Cancer

To gain insight into clinical response to platinum-based chemotherapy (PBC) in NSCLC, matched tumor and nontumor lung tissues from PBC-treated NSCLC patients – nonresponders as well as non-responders – and tumor tissue from an independent test set were profiled using microarrays (Petty et al. 2006). Lysosomal protease inhibitors SerpinB3 and cystatin C were highly correlated with clinical response and were further evaluated by immunohistochemistry in PBC-treated patients. This pathway within tumor cells, not previously suspected to be involved in lung cancer, was shown to cause resistance to chemotherapy, thus preventing the PBC from killing the cancer cells. This finding has led to the development of a new test that may allow clinicians to predict whether or not a lung cancer patient will respond to chemotherapy and help in decision-making about how the patient could best be treated, therefore, moving lung cancer patients closer to personalized treatments. This finding could also pave the way for the development of new drugs to target this pathway, which could subsequently lead to more effective treatments for lung cancer.

**Polymorphisms in the MDR1 Gene** These may have an impact on the expression and function of P-glycoprotein encoded by it, thereby influencing the response to chemotherapy. Patients harboring the 2677G-3435C haplotype have a statistically significant better response to chemotherapy compared to those with the other haplotypes combined. Therefore, MDR1 polymorphisms can be used for predicting treatment response to etoposide-cisplatin chemotherapy in SCLC patients.

**NTRK1 Oncogene Fusions** A novel class of oncogenes, NTRK1 fusions, were detected in lung adenocarcinomas by NGS or FISH (Doebele et al. 2013). Additional studies to determine the frequency and characteristics of NTRK1 fusions in lung cancer are ongoing. These findings suggest that prospective clinical trials of Trk inhibitors in NTRK1 fusion positive patients may be warranted.

### Testing for Prognosis of Lung Cancer

A substantial number of studies have reported the development of gene expression-based prognostic signatures for lung cancer. The ultimate aim of such studies should be the development of well-validated clinically useful prognostic signatures that improve therapeutic decision making beyond current practice standards. A review of published articles on gene expression based prognostic signatures in lung cancer reveals little evidence that any of the signatures are ready for clinical use.

Life Technologies' Pervenio™ Lung RS (originally Pinpoint Dx Lung™ Assay developed by Pinpoint Genomics Inc), a 14-gene expression assay that uses real-time qPCR and runs on FFPE tissue samples to differentiate patients with heterogeneous statistical prognoses, has been developed for patients with non-squamous NSCLC (Kratz et al. 2012). Among patients whom the assay identified as low-risk for recurrence, 71.4 % were still alive after 5 years, and among patients it determined to be at intermediate risk for recurrence 58.3 % survived >5 years. Among high-risk patients, 49.2 % survived >5 years. It reliably identifies patients with early-stage non-squamous NSCLC at high risk for mortality after surgical resection, and it provides prognostic differentiation of patients with early-stage disease and might be helpful in the identification of the most appropriate application of treatment guidelines to improve clinical outcomes. Life Technologies' CLIA laboratory, obtained through the acquisition of Navigenics, is licensed in the US and has currently validated Pervenio™ in most states.

Sixteen genes that correlated with survival among patients with NSCLC were identified by analyzing microarray data and risk scores (DUSP6, MMD, STAT1, ERBB3, and LCK) were selected for RT-PCR and decision-tree analysis (Chen et al. 2007). The five-gene signature is closely associated with relapse-free and overall survival among patients with NSCLC.

## *Personalized Management of Malignant Melanoma*

The incidence of melanoma is rising at an alarming rate and has become an important public health concern. If detected early, melanoma carries an excellent prognosis after appropriate surgical resection. Unfortunately, advanced melanoma has a poor prognosis and is notoriously resistant to radiation and chemotherapy. There are few effective therapies for metastatic melanoma. The two therapies approved by the FDA, high dose IL-2 and dacarbazine, are each associated with response rates of 10–20 % and a small percentage of complete responses; neither improves overall survival. The relative resistance of melanoma to a wide-range of chemotherapeutic agents and high toxicity of current therapies has prompted a search for effective alternative treatments that would improve prognosis and limit side effects.

Personalized medicine has long been a mainstay of the treatment of localized melanoma, involving surgical decisions that are individualized on the basis of measured differences as small as 0.01 mm, as well as other biomarkers of metastatic

potential, such as the presence of ulceration or mitoses. The genetic characterization of primary tumors as well as hereditary susceptibility to melanoma opens the door for tailored pharmacologic therapy. Genetic testing for CDKN2A and CDK4 are already available. Genetic tests for ARF and MC1R are likely to be available soon to evaluate an individual's hereditary risk for developing melanoma.

Several pharmacogenomic-based therapies are in development for melanoma. However, once melanoma spreads beyond the regional nodes, the lack of validated molecular targets hampers efforts to individualize therapy. In the past decade, targeted inhibitors have been developed for metastatic melanoma to enable more personalized therapies of genetically characterized tumors. The identification of somatic mutations in the gene encoding the serine-threonine protein kinase BRAF in the majority of melanomas offers an opportunity to test oncogene-targeted therapy for this disease.

### Inhibitors of BRAF Mutation for Metastatic Melanoma

Mucosal and acral-lentiginous melanomas, comprising 3 % of all melanomas, frequently harbor activating mutations of c-kit and drugs targeting this mutation seem to confer similar benefits for these types of tumors (Puzanov and Flaherty 2010).

V600E mutation of the BRAF serine/threonine kinase is present in >50 % of all melanomas. The mutation appeared to confer a dependency by the melanoma cancer cell on activated signaling through mitogen-activated protein kinase pathway. The frequency and location of this mutation (>95 % of all BRAF mutations being at V600 position) suggested its importance in melanoma pathophysiology and potential as a target for therapy. Vemurafenib (PLX4032, Plexxikon/Roche) is an orally available inhibitor of mutated BRAF. A phase II clinical trial showed that treatment of metastatic melanoma with vemurafenib in patients with tumors that carry the V600E BRAF mutation resulted in complete or partial tumor regression in the majority of patients (Flaherty et al. 2010). A phase III randomized clinical trial vemurafenib in patients with previously untreated, metastatic melanoma with the BRAF V600E mutation showed improved survival as compared to those treated with dacarbazine (Chapman et al. 2011). A treatment strategy that combines vemurafenib with Yervoy (Bristol-Myers Squibb), an approved melanoma treatment, will further improve outcomes for melanoma patients with BRAF mutation. In a phase III open-label randomized trial, trametinib, as compared with chemotherapy, improved rates of progression-free and overall survival among patients who had metastatic melanoma with a BRAF V600E or V600K mutation (Flaherty et al. 2012). Quest offers Roche's Cobas 4800 BRAF V600 Mutation Test that gauges which melanoma patients have BRAF V600E mutations and may receive treatment with another skin cancer agent, Zelboraf (vemurafenib). Quest also markets a Sanger sequencing-based laboratory-developed test for assessing BRAF mutations in melanoma patients.

In 2013, the FDA approved Tafinlar (dabrafenib) and Mekinist (trametinib) as treatments for advanced or unresectable melanoma patients whose tumors harbor

certain BRAF mutations. Simultaneously, the agency approved BioMérieux's THxID-BRAF companion diagnostic, a real-time PCR assay designed to identify patients whose tumors harbor BRAF V600E and BRAF V600K mutations, and thus are best responders to these agents. Approximately half of melanoma patients have tumors driven by BRAF mutations.

BRAF inhibitor Sorafenib has not shown selective affinity against tumors carrying BRAF mutations in clinical trials, whether used alone or in combination with other chemotherapies. It is possible that non-BRAF side effects of sorafenib limit the likelihood of achieving drug concentrations that are high enough to inhibit V600 mutation.

### Management of Drug-Resistant Metastatic Melanoma

BRAF inhibitors Vemurafenib and Dabrafenib markedly inhibit tumor growth and advance patients' overall survival but this response is almost inevitably followed by complete tumor relapse due to drug resistance hampering the encouraging initial responses. Several mechanisms of resistance within and outside the MAPK pathway have now been uncovered and have paved the way for clinical trials of combination therapies to try and overcome tumor relapse. It is apparent that personalized treatment management will be required in this new era of targeted treatment. Circulating tumor cells (CTCs) provide an easily accessible means of monitoring patient relapse and several new approaches are available for the molecular characterization of CTCs. Thus CTCs provide a monitoring tool to evaluate treatment efficacy and early detection of drug resistance in real time. Advances in the molecular analysis of CTCs may provide insight into new avenues of approaching therapeutic options that would benefit personalized melanoma management (Klinac et al. 2013).

### Vaccine for Malignant Melanoma Based on Heat Shock Protein

Autologous tumor-derived HSP gp96 peptide complex (HSPPC-96, Prophage®, vitespen) vaccine (Agenus Inc) is emerging as a tumor- and patient-specific cancer vaccine, with confirmed activity in several malignancies. It has been tested in phase III clinical trials in advanced melanoma with evidence for efficacy in patients with earlier stage disease. HSPPC-96-based vaccine demonstrated an excellent safety profile, thus emerging as a novel therapeutic approach with a suggestive role in cancer therapy. Further investigations are needed to understand the biological basis of immune functions in order to improve the clinical outcome of HSP-based cancer therapy. In the near future, the combination of HSP-based vaccines with other biological compounds might represent a successful strategy in the therapy of advanced melanoma.

## *Personalized Management of Pancreatic Cancer*

Pancreatic cancer is the fourth leading cause of death from cancer in the US; approximately 95 % of those affected die from it. The lifetime risk of developing pancreatic cancer is about 1 in 71. There are two types of pancreatic cancer: exocrine tumors and neuroendocrine tumors. Exocrine tumors are the majority of pancreatic cancers, and the most common form is an adenocarcinoma, which begin in gland cells, usually in the ducts of the pancreas. These tumors tend to be more aggressive than neuroendocrine tumors, but if detected early enough they can be treated effectively with surgery. Neuroendocrine tumors constitute only 1 % of all pancreatic cancers. They can be benign or malignant, but the distinction is often unclear and sometimes apparent only when the cancer has spread beyond the pancreas. The 5-year survival rate for neuroendocrine tumors can range from 50 % to 80 %, compared with less than 5 % for adenocarcinoma. Pancreatic cancer is so lethal because during the early stages, when it would be most treatable, there are usually no symptoms. It tends to be discovered at advanced stages when abdominal pain or jaundice may result. More advanced tumors have a higher risk of recurrence, and can spread to the liver. Pancreatic cancer is usually controllable only through removal by surgery, and only if found before it has spread. Palliative care can help a patient's quality of life if the disease has spread. The survival rate of pancreatic cancer patients is the lowest among those with common solid tumors, and early detection is one of the most feasible means of improving outcomes. Currently there are no general screening tools.

Two drugs are approved for treatment of pancreatic neuroendocrine tumors: everolimus (Novartis' Afinitor), and sunitinib malate (Pfizer's Sutent), which suppress angiogenesis and metabolism of the tumor cells. This is a progress compared to previous standard of care, which was chemotherapy, but both these drugs can have severe adverse effects. A number of new agents are being looked at in clinical trials that focus on pathways involved in pancreatic cancer. One is an antibody in development by NCI that blocks the protein PD-1 on the surface of pancreatic cancer, and would be more effective because it would produce an enhanced immune response against the tumor. Targeted nanoparticles coated with material that hone in on tumor cells and deliver drugs to kill them are being tested in animal models as treatment for metastatic neuroendocrine tumors. The main advantage would be reducing the toxicity of the drugs to the normal tissues of the body. The future treatment of pancreatic cancer will involve a personalized approach, i.e. matching a patient's particular type of tumor with treatment using genomic information.

### Biomarkers of Pancreatic Cancer

Unlike screenings for other conditions such as colon, breast and prostate cancers, there is no routine way to see whether a patient has a tumor in the pancreas. Current research is focused on finding biomarkers of pancreatic cancer so that a simple blood or urine test could be developed. Because of the complex pathophysiology of

pancreatic cancer, sensitive and specific biomarkers are also required. Extensive genomics/transcriptomics and proteomics studies are being carried out to find candidate biomarkers and contribute to high-throughput systems for large cohort screening. Among numerous biomarkers histone modifications are promising indicators of prognosis and response to therapy.

### Histone Modifications Predict Treatment Response in Pancreatic Cancer

Measuring levels of specific histone modifications within cells has previously shown that low cellular levels of particular histones could determine which prostate cancer patients were more likely to suffer a recurrence and which patients with lung and kidney cancers would experience poorer survival rates. An assay to detect histone modifications can now be used to predict prognosis and response to treatment in subsets of patients with pancreatic cancer (Manuyakorn et al. 2010). The scientists used tissues from a cohort of patients enrolled in the radiation therapy oncology group (RTOG) 9704 trial, a multicenter, phase III study of pancreatic cancer comparing adjuvant gemcitabine with 5-FU, and a separate cohort of patients with stage 1 or 2 pancreatic cancer. Immunohistochemistry was performed for histone H3 lysine 4 dimethylation (H3K4me2), histone H3 lysine 9 dimethylation (H3K9me2), and histone H3 lysine 18 acetylation (H3K18ac). Positive tumor cell staining for each histone modification was used to classify patients into low- and high-staining groups, which were related to clinicopathological parameters and clinical outcome measures. Low cellular levels of H3K4me2, H3K9me2, or H3K18ac were each significant and independent predictors of poor survival. Combined low levels of H3K4me2 and/or H3K18ac were the most significant predictor of overall survival. In subgroup analyses, histone levels were predictive of survival specifically for those patients with node-negative cancer or for those patients receiving adjuvant 5-FU but not gemcitabine in RTOG 9704. The investigators concluded that cellular levels of histone modifications define previously unrecognized subsets of patients with pancreatic adenocarcinoma with distinct epigenetic phenotypes and clinical outcomes and represent prognostic and predictive biomarkers that could form basis of clinical decisions, including the use of 5-FU chemotherapy. Further research in cell lines and animal models will determine what, if any, role the histone modifications have in causing the development of aggressive forms of pancreatic cancer. Uncovering the mechanism of how the histone modifications are associated with cancer development and/or progression may facilitate design of strategies to interfere with that process and form the basis for a targeted therapy or chemoprevention.

### Transport Properties of Pancreatic Cancer and Gemcitabine Delivery

The therapeutic resistance of pancreatic ductal adenocarcinoma (PDAC) is partly ascribed to ineffective delivery of chemotherapy to cancer cells. To study this problem, a method has been developed to measure mass transport properties during

routine contrast-enhanced CT scans of individual human PDAC tumors (Koay et al. 2014). Additionally, gemcitabine infusion during PDAC resection was evaluated in patients, measuring gemcitabine incorporation into tumor DNA and correlating its uptake with human equilibrative nucleoside transporter (hENT1) levels, stromal reaction, and CT-derived mass transport properties. Study of associations between CT-derived transport properties and clinical outcomes in patients who received preoperative gemcitabine-based chemoradiotherapy for resectable PDAC revealed striking differences in transport properties between normal pancreas and the tumor. Reflecting the interpatient differences in contrast enhancement, resected tumors exhibited dramatic differences in gemcitabine DNA incorporation, despite similar intravascular pharmacokinetics. Gemcitabine incorporation into tumor DNA was inversely related to CT-derived transport parameters and PDAC stromal score, after accounting for hENT1 levels. Moreover, stromal score directly correlated with CT-derived parameters. Among patients who received preoperative gemcitabine-based chemoradiotherapy, CT-derived parameters correlated with pathological response and survival. It was concluded that gemcitabine incorporation into tumor DNA is highly variable and correlates with multiscale transport properties that can be derived from routine CT scans. Furthermore, pretherapy CT-derived properties correlate with clinically relevant endpoints. The study introduces and strongly supports the concept of quantitative biophysical markers that may provide clinically useful data to help direct personalized cancer treatment and thereby improve the survival of patients with PDAC and other solid tumors.

## *Personalized Management of Prostate Cancer*

Prostate cancer is the most common type of cancer found in American men, other than skin cancer, and is the second leading cause of cancer deaths, according to the American Cancer Society. Over 240,000 men are diagnosed with prostate cancer in the US every year. Research is aimed at finding gene variants associated with susceptibility to cancer. Molecular diagnostics has been used to guide therapy of prostate cancer.

### Assessing Susceptibility to Prostate Cancer by Genotyping

The HOXB13 gene has been implicated in prostate cancer (PrCa) susceptibility. High resolution fine-mapping analysis has been performed to comprehensively evaluate the association between common genetic variation across the HOXB genetic locus at 17q21 and PrCa risk (Saunders et al. 2014). This involved genotyping 700 SNPs using a custom Illumina iSelect array (iCOGS) followed by imputation of 3195 SNPs in 20,440 PrCa cases and 21,469 controls in The PRACTICAL consortium. The study identified a cluster of highly correlated common variants situated within or closely upstream of HOXB13 that were significantly associated

with PrCa risk, described by rs117576373. Additional genotyping, conditional regression and haplotype analyses indicated that the newly identified common variants tag a rare, partially correlated coding variant in the HOXB13 gene (G84E, rs138213197), which has been identified as a moderate penetrance PrCa susceptibility allele. The potential for genome-wide associations detected through common SNPs to be driven by rare causal variants with higher relative risks has long been proposed; however, this is the first experimental evidence for this phenomenon of synthetic association contributing to cancer susceptibility. Synthetic associations at genome-wide signals could therefore account for a proportion of the missing heritability of complex diseases such as cancer. Such findings are useful for understanding some of the biological underpinnings of prostate cancer risk, but there is possibility of finding other situations involving synthetic associations between common and rare variants influencing specific traits or conditions.

**Diagnostics for Guiding Therapy of Prostate Cancer**

Testing tissues from men with prostate cancer has demonstrated how loss of PTEN, a gene that inhibits tumor growth, results in the uncontrolled activation of a tumor promoting protein, AKT. AKT then activates the enzyme mTOR, which subsequently activates S6. This is the basis of a tumor promoting cascade, similar to a domino effect. These biomarkers can be used to predict response to an experimental therapy known as CCI-779, an inhibitor of mTOR. A drug that inhibits mTOR should impact the tumor cells but have no effect on the normal cells. When mTOR is inhibited, the cascade comes to a standstill and tumors stop growing. Prior to identifying this method, there was no molecular method to predict which men with prostate cancers would be sensitive to CCI-779. Now oncologists can customize "targeted" cancer treatments for each patient based on the molecular make-up of their tumors. These "smart drugs" selectively stop the growth of tumor cells with the molecular abnormality. Of those, about 25–30 % were predicted to have tumors that are missing PTEN. Therefore, the experimental drug could potentially help about 60,000 prostate cancer patients a year, if the laboratory results are confirmed in clinical trials, which are ongoing.

Prostate Px (Aureon Laboratories), integrates histology, molecular biology and clinical information and applies bioinformatics to stratify patients as high or low risk for disease recurrence post-prostatectomy. Results are provided as the Prostate Px score (0–100), which reports the likelihood of recurrence of the prostate cancer. In a prospective study, integration of clinicopathological variables with imaging and biomarker data (systems pathology) resulted in a highly accurate tool for predicting clinical failure within 5 years after prostatectomy (Donovan et al. 2008). The data support a role for androgen receptor signaling in clinical progression and duration of response to androgen deprivation therapy.

The anticancer agent docetaxel and thalidomide shows significant inter-individual variation in their pharmacokinetic and toxicity profiles as well as wide pharmacological variations. In one study, patients with prostate cancer enrolled in a

randomized phase II trial using docetaxel and thalidomide versus docetaxel alone were genotyped using the Affymetrix DMET 1.0 platform, which tests for 1,256 genetic variations in 170 drug disposition genes (Deeken et al. 2010). Genetic polymorphisms were analyzed for associations with clinical response and toxicity. In all, 10 SNPs in three genes were potentially associated with response to therapy: peroxisome proliferator-activated receptor-δ (PPAR-δ), sulfotransferase family, cytosolic, 1C, member 2 (SULT1C2) and carbohydrate (chondroitin 6) sulfotransferase 3 (CHST3). Genotyping results between drug metabolizing enzymes and transporters (DMET) and direct sequencing showed >96 % of concordance. These findings highlight the role that non-CYP450 metabolizing enzymes and transporters may have in the pharmacology of docetaxel and thalidomide. DMET appears to offer great promise in this field as a reliable test unveiling genetic variations that correlated with drug effectiveness and toxicity.

### Detection of Prostate Cancer Metastases

Prostate circulating tumor cells (PCTCs) in circulation are shed from either a primary tumor or metastases, which are directly responsible for most prostate cancer deaths. Quantifying exfoliated PCTCs may serve as an indicator for the clinical management of prostate cancer, isolating and removing of PCTCs could potentially reduce prostate cancer metastasis, and culturing and characterizing captured PCTCs could facilitate the development of personalized treatment options. PSMA, an established biomarker for prostate cancer, is strongly expressed on prostate tumor cells associated with high-grade primary, androgen-independent, and metastatic tumors.

Chemoaffinity capture with magnetic beads of pre-targeted PCTCs from peripheral blood can serve as an effective tool for the detection of metastatic prostate cancer, monitoring of treatment, and the development of personalized therapy based on the responsiveness of PCTCs to chemotherapeutic strategies (Wu et al. 2012). MenaCalc™ Prostate (MetaStat Inc) is a diagnostic for prostate cancer to help in informed decision about whether to undergo radical surgery and risk its dreaded side effects.

### Early Detection of Cancer Recurrence and Guiding Treatment

An automated gold nanoparticle bio-barcode assay probe has been described for the detection of prostate specific antigen (PSA) at 330 fg/mL, along with the results of a clinical pilot study designed to assess the ability of the assay to detect PSA in the serum of 18 men who have undergone radical prostatectomy for prostate cancer (Thaxton et al. 2009). Available PSA immunoassays are often not capable of detecting PSA in the serum of men after radical prostatectomy. This new bio-barcode PSA assay is approximately 300 times more sensitive than commercial immunoassays and all patients in this study had a measurable serum PSA level after radical

prostatectomy. Because the patient outcome depends on the level of PSA, this ultrasensitive assay enables: (1) informing patients, who have undetectable PSA levels with conventional assays, but detectable and nonrising levels with the barcode assay, that their cancer will not recur; (2) earlier detection of recurrence earlier because of the ability to measure increasing levels of PSA before conventional tools can make such assignments; and (3) use of PSA levels, which would otherwise not be detectable with conventional assays, to follow the response of patients to treatment.

### Effects of Lifestyle Changes Shown by Gene Expression Studies

Epidemiological and prospective studies indicate that comprehensive lifestyle changes may modify the progression of prostate cancer. A pilot study was conducted to examine changes in prostate gene expression in a unique population of men with low-risk prostate cancer who declined immediate surgery, hormonal therapy, or radiation and participated in an intensive nutrition and lifestyle intervention while undergoing careful surveillance for tumor progression (Ornish et al. 2008). Consistent with previous studies, significant improvements in weight, abdominal obesity, blood pressure, and lipid profile were observed. Gene expression profiles were obtained from RNA samples from control prostate needle biopsy taken before intervention to RNA from the same patient's 3-month postintervention biopsy. Quantitative real-time PCR was used to validate array observations for selected transcripts. Two-class paired analysis of global gene expression using significance analysis of microarrays detected 48 up-regulated and 453 down-regulated transcripts after the intervention. Pathway analysis identified significant modulation of biological processes that have critical roles in tumorigenesis, including protein metabolism and modification, intracellular protein traffic, and protein phosphorylation. Intensive nutrition and lifestyle changes may modulate gene expression in the prostate. Understanding the prostate molecular response to comprehensive lifestyle changes may strengthen efforts to develop effective prevention and treatment. The study not only provides insights into potential drug targets, but also suggests that lifestyle changes could produce benefits akin to therapeutic interventions. Larger clinical trials are warranted to confirm the results of this pilot study.

### Prolaris Assay for Determining Prognosis in Prostate Cancer

Prolaris test (Myriad) quantifies a patient's risk of disease progression and prostate cancer specific mortality using a gene-expression-based cell cycle progression (CCP) score. A prospective study found that CCP score adds meaningful new information to risk assessment for localized prostate cancer patients (Shore et al. 2014). Use of the test in practice is likely to have an impact on the management in a significant portion of tested patients, particularly by shifting the trend towards more conservative management. This could reduce overtreatment of patients with less aggressive disease, decreasing patient morbidity and costs for payers and the healthcare system.

**Personalized Peptide Vaccine for Prostate Cancer**

HER2/neu protein is also expressed in prostate cancer. High-risk prostate cancer (HRPC) patients demonstrating varying levels of HER2/neu expression have vaccinated with E75 peptide plus GM-CSF to prevent postprostatectomy PSA and clinical recurrences. In a prospective study HER2/neu (E75) vaccine was shown to prevent or delay recurrences in HRPC patients if completed before PSA recurrence (Gates et al. 2009). A phase I clinical trial of Ii-Key/HER-2/neu hybrid peptide vaccine with recombinant GM-CSF as adjuvant in patients with HER-2/neu positive prostate cancer showed that the vaccine is safe and can induce HER-2/neu-specific cellular immune responses in patients with castrate-sensitive and castrate-resistant prostate cancer (Perez et al. 2010).

A randomized phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP has been conducted in patients with castration resistant prostate cancer (Noguchi et al. 2010). The combined therapy was well tolerated with increased levels of IgG and cytotoxic-T cell responses to the vaccinated peptides and resulted in an improvement of progression free survival as compared to the standard-dose EMP alone.

### *Personalized Management of Thyroid Cancer*

Personalized medicine has a potential for the management of patients with differentiated thyroid cancer (DTC). Majority of patients with DTC have a good prognosis. Nevertheless the outcome can be optimized by individualization, of the extent of surgery, the dosage of $^{131}$I therapy and the use of levothyroxine therapy (Luster et al. 2014). Newer imaging techniques and targeted molecular therapies such as multitargeted kinase inhibitors provide new options for the personalized care of patients with advanced disease for whom no effective therapies were available previously. Individualized therapies could reduce adverse effects, including the sometimes debilitating hypothyroidism that used to be required before initiation of $^{131}$I treatment, and major salivary gland damage, a common and unpleasant side effect of $^{131}$I therapy. Highly individualized interdisciplinary treatment of patients with DTC might lead to improved outcomes with reduced severity and frequency of complications and adverse effects. However, in spite of ongoing research, personalized therapies remain in their infancy.

## Future of Cancer Therapy

There are now unprecedented opportunities for the development of improved drugs for cancer treatment. Most of the genes in the majority of common human cancers are expected to be defined over the next 5 years. This will provide the opportunity to develop a range of drugs targeted to the precise molecular abnormalities that

drive various human cancers and will open up the possibility of personalized therapies targeted to the molecular pathology and genomics of individual patients and their malignancies. The new molecular therapies should be more effective and have less-severe side effects than cytotoxic agents. To develop the new generation of molecular cancer therapeutics as rapidly as possible, it is essential to harness the power of a range of new technologies. These include: genomic and proteomic methodologies (particularly gene expression microarrays); robotic high-throughput screening of diverse compound collections, together with in silico and fragment-based screening techniques; nanobiotechnology; new structural biology methods for rational drug design (especially high-throughput X-ray crystallography and NMR); and advanced chemical technologies, including combinatorial and parallel synthesis.

## *Challenges for Developing Personalized Cancer Therapies*

Two major challenges to cancer drug discovery are: (1) the ability to convert potent and selective lead compounds with activity by the desired mechanism on tumor cells in culture into agents with robust, drug-like properties, particularly in terms of pharmacokinetic and metabolic properties; and (2) the development of validated pharmacodynamic endpoints and molecular markers of drug response, ideally using noninvasive imaging technologies.

Many variables besides genotypes of patients would need to be considered in development of personalized therapies for cancer. An example of this is limitation of genotyping for methylenetetrahydrofolate reductase (MTHFR), which plays a central role in the action of 5-FU, an inhibitor of thymidylate synthase, by converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two polymorphisms in the MTHFR gene (677C>T and 1,298 A>C) have been considered as genomic predictors of clinical response to fluoropyrimidine-based chemotherapy (in combination with irinotecan or oxaliplatin). Results of studies on patients with metastatic CRC who have undergone 5-FU-containing chemotherapy as a first line treatment indicate that the MTHFR genotype cannot be considered as an independent factor of outcome.

## *Cancer Genome Atlas*

The Cancer Genome Atlas (TCGA) is a coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing (http://cancergenome.nih.gov/). TCGA is a joint effort of the NCI and the National Human Genome Research Institute (NHGRI), which are both part of the NIH. The Pilot Project focuses on three types of cancers: brain (glioblastoma multiforme), lung (squamous

carcinoma), and ovarian (serous cystadenocarcinoma). Together, these cancers account for more than 258,480 cancer cases each year in the US.

The Cancer Genome Characterization Centers support TCGA in accelerating the understanding of the molecular basis of cancer. A component of TCGA Pilot Project will be high-throughput genomic sequencing. This activity will be conducted by Genome Sequencing Centers that have extensive experience in large-scale genomic DNA sequencing.

There is a need for better description of the genetic damage that drives human cancers; this will form the basis for all future studies of cancer in the laboratory and the clinic and will provide immediate benefit for molecular diagnosis of human cancers as a basis for the development of personalized treatment of cancer.

## *COLTHERES Consortium*

COLTHERES (Colon Cancer and Therapeutics) is a consortium of EU-clinical centers and translational researchers who have received a total of €6.5 million of core funding from the FP7 organization to define and perform biomarker driven clinical trials to improve colon cancer therapy outcomes. It is a 4-year program that has used comprehensive molecularly-annotated colon cancers to define specific biomarkers of response or resistance to signaling pathway agents. The consortium is open to any pharmaceutical developer who wishes to determine which patients are most likely to respond to their novel cancer therapy and perform rapid proof-of-concept clinical trials. It is expected that the program will generate up to 100 new X-MAN™ (gene X-Mutant And Normal) genetically-defined human cell lines; accurately incorporating key biomarkers that are predicted to cause resistance to new targeted therapies. These cell lines will be owned by Horizon Discovery Ltd, which forms part of the Company's strategy to generate at least 2,500 new X-MAN models in 5-years. These models will support drug discovery researchers to understand how complex genetic diseases manifest themselves in real patients and help rationalize many aspects of drug development, and therefore the cost of bringing to market new personalized therapies.

Up to date () information on COLTHERES project can be viewed on the web site (http://www.coltheres.org/). The latest clinical trial (as of December 2014) in this project involves dacomitinib and MEK inhibitor PD-0325901 (Pfizer) in KRAS mutant CRC.

## *Computer and Imaging Technologies for Personalizing Cancer Treatment*

The Cancer Institute of New Jersey and IBM have collaborated to develop more accurate diagnostic tools aimed at improving cancer treatments and outcomes. They are using advanced computer and imaging technology to create a database where

physicians and scientists can compare patients' tissues with digitally archived cancerous tissues for which genomic and proteomic data is available. This will not only lead to more personalized treatment, but will also enhance cell and radiological cancer studies. The initiative was funded by grant from the NIH as an extension of the 2006 "Help Defeat Cancer" campaign. For that project, researchers used IBM's World Community Grid – a virtual supercomputer based on unused computer time donated by volunteers – to create an expression signature library for breast, colon, head, and neck cancers and to develop reliable analytical tools for high-throughput tissue microarrays. In the next phase, the project will expand into other types of cancer and also create a Center for High-Throughput Data Analysis for Cancer Research. The Center will rely on pattern recognition algorithms for developing diagnostic tools based on archived cancer specimens and radiology images. That information will be integrated with proteomic and genomic data to aid treatment recommendations. Several other institutions, including Rutgers University, Arizona State University, Ohio State University, and the University of Pennsylvania are involved in the project. IBM has donated high-performance systems to the Center, which use grid technology that allows collaborators from around the US access the Center's database and software.

## *Genomic Cancer Care Alliance*

Genomic Cancer Care Alliance – which currently involves founding organizations Fox Chase Cancer Center, Scripps Genomic Medicine, Omicia, El Camino Hospital, and the Translational Genomics Research Institute – launched a pilot study in 2010 to investigate the ability of whole-genome sequencing to guide treatment for patients who have responded poorly to initial therapy. The alliance is primarily funded by Life Technologies at present and uses the company's SOLiD 4 sequencing platform.

## *Integrated Genome-Wide Analysis of Cancer for Personalized Therapy*

An integrated genome-wide analysis of CNV in breast and colorectal cancers using approaches that can reliably detect homozygous deletions and amplifications such as SNP analysis and digital karyotyping, has revealed that the number of genes altered by major CNVs, deletion of all copies or amplification to at least a dozen copies per cell (Leary et al. 2008). This study has identified genes and cellular pathways affected by both CNVs and point alterations. Pathways enriched for genetic alterations included those controlling cell adhesion, intracellular signaling, DNA topological change, and cell cycle control. A comprehensive picture of genetic alterations in human cancer should therefore include the integration of sequence-based alterations together with copy number gains and losses. Combining copy

number and sequence data also holds promise for determining whether particular point mutations have a functional effect, the researchers noted. For example, if a gene turns up with a deletion in one sample and a point mutation in another, it could indicate that that point mutation is inactivating. Incorporating information on other genome-wide changes such as translocations and epigenetic changes could provide even greater insight into cancer, as will trying to determine the timing with which genetic alterations occur in cells. These analyses could prove useful for cancer personalizing diagnosis and therapy. For example, two-thirds of the breast and colorectal samples tested in the study contain alterations to four key signaling pathways, suggesting that drugs targeting these pathways could prove useful for treating both breast and colorectal cancers. Since several breast cancer samples tested contained changes to DNA topological pathways, some of these tumors may be candidates for topoisomerase-targeted therapies.

### *International Cancer Genome Consortium*

In 2008, Research organizations from around the world launched the International Cancer Genome Consortium (ICGC), which will have an impact on personalized management of cancer. ICGC aims to generate high-quality genomic data on up to 50 types of cancer through efforts projected to take up to a decade. The web site (http://www.icgc.org/) displays ICGC White Paper, detailing its policies and guidelines. ICGC invites research organizations in all nations. Current ICGC members include:

- Australia: National Health and Medical Research Council (Observer Status)
- Canada: Genome Canada; Ontario Institute for Cancer Research
- China: Chinese Cancer Genome Consortium
- Europe: European Commission (Observer Status)
- France: Institute National du Cancer
- India: Department of Biotechnology, Ministry of Science & Technology
- Japan: RIKEN; National Cancer Center
- Singapore: Genome Institute of Singapore
- United Kingdom: The Wellcome Trust; Wellcome Trust Sanger Institute
- United States: NIH

Each ICGC member intends to conduct a comprehensive, high-resolution analysis of the full range of genomic changes in at least one specific type or subtype of cancer, with studies built around common standards of data collection and analysis. Each project is expected to involve specimens from 500 patients and have an estimated cost of $20 million. As part of its coordination efforts, the ICGC will generate a list of 50 cancer types and subtypes that are of clinical significance around the globe. ICGC members plan to assume responsibility for specific cancers, and one of the ICGC's roles would be to facilitate the exchange of information to avoid duplication of participants' efforts. The ICGC's main criteria for prioritizing cancer types include: impact, incidence, age of onset, mortality rates, and availability of

therapies; scientific interest; and the ability to obtain enough high-quality samples to conduct a large-scale project.

To facilitate comparisons among different types of cancer, the ICGC guidelines list key factors for its members to consider in the production of genomic catalogs. Those factors include comprehensiveness, which involves detecting all cancer-related genetic mutations that occur in at least 3 % of tumor samples; resolution, which involves generating data at the level of individual DNA bases; quality, which involves monitoring based on common standards for pathology and technology; and controls, which involves comparisons of data from matched, noncancerous tissue.

ICGC member nations will agree to common standards for informed consent and ethical oversight. Although the informed consent process will necessarily differ according to each member country's requirements, the consortium's policies state that cancer patients enrolled in an ICGC-related study should be informed that their participation is voluntary, that their clinical care will not be affected by their participation and that data obtained from analyses using their samples will be made available to the international research community. ICGC members also should take steps to ensure that all samples will be coded and stored in ways that protect the identities of the participants. To maximize the public benefit from ICGC member research, data will be made rapidly available to qualified investigators. All consortium participants agree not to file any patent applications or make intellectual property claims on primary data from ICGC projects.

Currently (as of the end of 2014), the ICGC has received commitments from funding organizations in Asia, Australia, Europe, North America and South America for 74 project teams in 17 jurisdictions to study >25,000 tumor genomes. Projects that are currently funded are examining tumors affecting: the biliary tract, bladder, blood, bone, brain, breast, cervix, colon, eye, head and neck, kidney, liver, lung, nasopharynx, oral cavity, ovary, pancreas, prostate, rectum, skin, soft tissues, stomach, thyroid and uterus. The genomic analyses of tumors conducted by ICGC members in Australia (ovarian and pancreatic cancer), Canada (pancreatic, pediatric brain and prostate cancer), China (bladder, esophageal, gastric and renal cancer), European Union/France (renal cancer), France (liver cancer), Germany (blood, brain and prostate cancer), India (oral cancer), Japan (liver cancer), Saudi Arabia (thyroid cancer), South Korea (blood and lung cancer), Spain (blood cancer), the UK (blood, bone, breast, esophageal, lung, prostate and skin cancer) and the USA (bladder, blood, brain, breast, cervical, colon, gastric, head and neck, liver, lung, ovarian, pancreatic, prostate, rectal, renal, skin, thyroid and uterine cancer) are now available through the Data Coordination Center housed on the ICGC website.

## *National Cancer Institute of US*

The NCI plans to fund projects for 2015 that cover the following areas:

- Compare recurrent and non-recurrent screen-detected lesions and interval cancers at the molecular level.

- Use knowledge from genome-wide association studies and chromosomal instability to predict the progression from benign to malignant cancers and develop molecular tests to identify genes associated with risk progression in early lesions.
- Use systems biology approaches to define the trajectories of different types of lesions and the corresponding risk of clinically significant malignancies.
- Use whole-genome sequencing and somatic gene alterations to develop phylogenies to infer the genomic ancestry of lesions and to investigate premalignant conditions
- Analyze tumor heterogeneity using single cell analyses.

Up to $4.5 million will be provided to fund research teams in a network of 8–10 Molecular Characterization Laboratories with the aim of better predicting which lesions are progressive and require interventions and which are indolent. These teams will use a range of omics approaches to characterize lesions at the molecular and cellular levels. They will also establish a biospecimen repository to house screen-detected lesions and interval cancers. These projects will contribute to improving personalized cancer therapy.

## *PREDICT Consortium*

PREDICT (Personalized RNA Interference to Enhance the Delivery of Individualized Cytotoxic and Targeted Therapeutics Consortium) was created in 2009 to coordinate single drug clinical trials with personalized tumor functional genomic analysis to define patient-specific drug sensitivity pathways and biomarkers predictive of drug response. Partners in the consortium include Horizon Discovery Ltd, Technical University of Denmark, Cancer Research UK, the Welcome Trust Sanger Institute, Institut Gustave Rousey, The Royal Marsden NHS Trust, and Bayer Healthcare.

The consortium integrates expertise in renal carcinoma clinical trial recruitment, whole-genome sequencing technologies, ex vivo cancer cell line cultures, and personalized RNAi screening technologies. Research is supported by a grant that Horizon Discovery is sharing with the University of Torino Medical School to develop models of inherited and somatic genetic variation for research into new drugs and diagnostics for cancer.

## *Quebec Clinical Research Organization in Cancer*

Quebec Clinical Research Organization in Cancer (Q-CROC) conducts biomarker-driven clinical research into cancer with a public-private partnership and "Centre of Excellence PreThera Research." In April 2014, Merck Canada awarded a C$2 million (US$1.8 million) grant to Q-CROC in support of research into personalized medicine approaches to cancer. Q-CROC (http://www.qcroc.ca/en), a provincial interface for clinical research, aims to facilitate the sharing of information between industry, government, healthcare establishments, and the research community.

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# Chapter 11
# Personalized Management of Infectious Diseases

## Introduction

Personalized approach involves selection of an appropriate treatment right from the start for optimal effectiveness and for reduction of development of drug resistance. Advances in point-of-care (POC) diagnostics have enable physicians to avoid dispensing antibiotics for viral infections and fevers of unknown origin. Improved diagnostics can enable prescriptions according to a pathogen's susceptibilities. Similar to the concept of personalized medicine based on patients' genetic differences, treatment of infectious diseases involves individualizing therapy according to genetic differences in infectious agents. Various examples of personalized management antimicrobial therapeutics are given, antibacterial as well as antiviral.

## Personalized Management of Bacterial Infections

### *Bacterial Genomics and Sequencing*

Sequencing has been employed extensively for the study of bacterial genomics (Jain 2015b). Some examples of the role of sequencing in the personalized management of bacterial infections are given here.

#### Pyrosequencing of Microbial Flora in Leg Ulcers

Approximately 1 out of every 100 individuals has some form of venous insufficiency, which can lead to chronic venous disease and venous leg ulcer (VLU). There are known underlying pathologies which contribute to the chronic nature of VLU including biofilm phenotype infections. Pyrosequencing based approaches have been used to evaluate VLU to characterize their microbial ecology (Wolcott et al. 2009).

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Results show that VLU infections are polymicrobial with no single bacterium colonizing the wounds. The most ubiquitous and predominant organisms include various anaerobes, Staphylococcus, Corynebacterium, and Serratia. Topological analysis of VLU shows some notable differences in bacterial populations across the surface of the wounds highlighting the importance of sampling techniques during diagnosis. Metagenomics provide a preliminary indication that there may be protozoa, fungi and possibly an undescribed virus associated with these wounds.

The polymicrobial nature of VLU and previous research on diabetic foot ulcers and surgical site infections suggest that the future of therapy for such wounds lies in the core of the logical and proven multiple concurrent strategy approach, which has been termed "biofilm-based wound care" and the use of individualized therapeutics rather than a single treatment modality.

### Sequencing for Study of Antibiotic Resistance in Bacteria

Antibiotic resistance can gradually evolve through the sequential accumulation of multiple mutations. To study this evolution, scientists at Harvard Medical School have developed a selection device, the 'morbidostat', which continuously monitors bacterial growth and dynamically regulates drug concentrations to constantly challenge the evolving population. They analyzed the evolution of resistance in *E. coli* under selection with single drugs, including chloramphenicol, doxycycline and trimethoprim. Over a period of ~20 days, resistance levels increased dramatically, with parallel populations showing similar phenotypic trajectories. WGS of the evolved strains identified mutations both specific to resistance to a particular drug and shared in resistance to multiple drugs (Toprak et al. 2011). Chloramphenicol and doxycycline resistance evolved smoothly through diverse combinations of mutations in genes involved in translation, transcription and transport. In contrast, trimethoprim resistance evolved in a stepwise manner, through mutations restricted to the gene encoding the enzyme dihydrofolate reductase (DHFR). Sequencing of DHFR over the time course of the experiment showed that parallel populations evolved similar mutations and acquired them in a similar order.

### Sequencing for Predicting the Virulence of MRSA

Microbial virulence is a complex and often multifactorial phenotype, intricately linked to a pathogen's evolutionary trajectory. Toxicity, the ability to destroy host cell membranes, and adhesion, the ability to adhere to human tissues, are the major virulence factors of many bacterial pathogens, including *S. aureus*. A study assayed the toxicity and adhesiveness of 90 MRSA isolates and found that while there was remarkably little variation in adhesion, toxicity varied by over an order of magnitude between isolates, suggesting different evolutionary selection pressures acting on these two traits (Laabei et al. 2014). The authors performed a GWAS and identified a large number of loci, as well as a putative network of interacting loci that are significantly associated with toxicity. Despite this apparent complexity in regulation of toxicity, a

predictive model based on a set of significant SNPs and indels showed a high degree of accuracy in predicting an isolate's toxicity solely from the genetic signature at these sites. The results thus highlight the potential of using sequence data to determine clinically relevant parameters and for understanding the microbial virulence of MRSA.

## *Role of Rapid Molecular Diagnosis at Point of Care*

In medicine, quantitative measurement of specific strains of infectious organisms is very important in emergency situations because the physician must start therapy immediately if the patient is in critical condition. An effective test must be precise, rapid, and also measure the infectious burden. At the same time, better testing will quickly identify the organism's strain and drug susceptibility, reducing the delay in finding the right antibiotic.

Traditional diagnostic testing often requires several days to isolate and grow the infectious organism, and to test its sensitivity to specific antibiotics. Until then, the physician must use powerful broad-spectrum antibiotics. Widespread use of these antibiotics leads to the emergence of drug resistance, which then narrows the number of drugs available to treat serious infections. PCR-based tests for the <1 h detection and identification of infectious agents are being developed that will revolutionize the decision-making process of health care professionals.

Detection, identification, and characterization of pathogens is being revolutionized by the combination of the seemingly disparate fields of nucleic acid analysis, bioinformatics, data storage and retrieval, nanotechnology, physics, microelectronics, and polymer, solid state, and combinatorial chemistry. The first application of DNA chips in POC testing will probably be for identifying pathogens and their antimicrobial resistance potential. These developments, particularly with regard to POC testing, have important implications for the delivery of health care. It will be possible to miniaturize test kits, which can be swallowed or added to body fluids and coupled with data transmitters so that results can be sent to remote site for analysis. Rapid molecular diagnosis will improve the initial management of the patient, determine the need for isolation and help the selection of optimal antimicrobials if they are needed. Nanotechnology-based tests for detection of microorganisms are also in development. These refinements in diagnostic technologies will not only enable personalized management of infections but will also be an important factor in the control of emergence of microbial resistance and epidemics.

## *Personalized Vaccines Against Bacterial Infections*

There are inter-individual variations in immune response to commonly used prophylactic vaccines against infectious diseases, which are is influenced by sex, MHC, age and hormones status of individuals. Natural microbiota in the gastrointestinal tract appear to contribute to nearly every aspect of physiology of the host. It may be responsible for diverse vaccine efficacy observed in humans from developing

Manipulation of the microbiota by probiotics and/or prebiotics is a therapeutic as well as prophylactic strategy for many infectious and inflammatory diseases within the gut, but it may be also used for improving vaccine efficacy (Długońska and Grzybowski 2011).

## *Personalized Management of Sepsis*

Severe sepsis and septic shock are among the leading causes of death with mortality ranging between 35 % and 50 %. Adequate management of sepsis depends on early detection (earlier than conventional blood cultures) and early administration of appropriate antimicrobials. This can be achieved by molecular diagnostic techniques. Assessment of the immune status of the host should also be done faster than that possible by conventional biomarkers. Among assays of rapid identification of pathogens that have been grown in blood cultures, PNA-FISH is FDA approved for the diagnosis of enterococcal infections. SeptiFast (Roche Diagnostics) is a commercially available multiplex real-time PCR for simultaneous detection of DNA of 25 different bacterial and fungal species within a few hours. Other molecular diagnostics for sepsis are described in a special report (Jain 2015c).

There is more individual variability among septic patients than previously recognized. Pathophysiology of sepsis is a complex and dynamic process that originates from the host immune response to infection and varies according to the genetic predisposition, immune status and co-morbid conditions of the host, the type of pathogen and the site and extent of infection. Until now, efforts to stratify septic patients according to their immune profile were hampered by the lack of specific biomarkers. Advances in molecular medicine have enabled development of tools that will facilitate a faster and more precise diagnosis of infections. Individual variability between each patient's responses to infection can assist in tailoring therapeutic interventions to the individual's disease profile and monitoring treatment response. Gene profiling of the host is a promising approach because of the individualized nature of sepsis to enable personalized management (Kotsaki and Giamarellos-Bourboulis 2012). Advances in genomics will improve personalized approach to each septic patient.

## *Personalized Management of Tuberculosis*

Tuberculosis (TB) is a global pandemic that threatens to overwhelm healthcare budgets in many developing countries. It is estimated that at least eight million people develop active TB annually, of whom two million die. It has been the cause of a global health emergency for over 10 years owing to factors such as social stigma, patient compliance and lack of investment in a thorough TB control program. Despite the availability of adequate effective treatment, many patients default on

treatment, experience adverse side effects from antibiotics or fail to respond rapidly and recover. These factors have resulted in the worrying emergence of drug resistance, leading to multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of TB becoming prevalent. This is a particular problem in the developing world, where the majority of patients with TB also have HIV, making effective eradication extremely difficult.

Isoniazid, one of the most important first-line tuberculosis drugs, is acetylated in the liver to a variable degree in different individuals giving rise to fast, intermediate and slow acetylator phenotypes. Different genetic mutations may play a role in determining how a patient will respond to the commonly used TB medication isoniazid (Werely et al. 2007). Acetylation status of individuals plays an important contributory role in the tuberculosis pandemic. It is important to study the acetylation alleles, and to understand isoniazid metabolism and the manner in which it could affect patient compliance, isoniazid-toxicity and the emergence of drug-resistant strains of mycobacteria. These phenotypes have been linked to different genetic variants, primarily present in the NAT2 gene (see Chap. 3). The standard drug dose currently administered to patients, regardless of their acetylator status, may not be appropriate for certain people. Individualization of isoniazid therapy may help to prevent adverse drug reactions experienced by a small percentage of patients thought to be 'slow-acetylators' of the drug. Conversely fast-acetylators may not be receiving sufficient amounts of the drug to combat TB successfully, therefore increasing the likelihood of a relapse and development of drug resistance. Confirmation of the genetics of isoniazid metabolism by a simple test to determine acetylator status would be desirable and this should be available at the same laboratories that currently perform diagnostics for TB.

## Personalized Management of Viral Infections

Antiviral therapeutics is dealt with in detail in a special report on this topic (Jain 2015a). A schematic approach to integration of antiviral strategies is shown in Fig. 11.1.

Currently used therapies for viral infections such as HIV/AIDS and influenza target certain receptors or enzymes. Most of these are specific for each infection whereas others such as protease inhibitors can be used in more than one type of infection. None of these approaches cover multiple receptors. Efficacy is limited and there are problems with development of resistance, particularly in case of HIV.

Ligand-binding epitopes of proteins can mutate rapidly, as shown by viral mutations that lead to escape from neutralizing antibodies. An approach, dubbed "checkmate analysis," may predict which antibodies or small molecule therapeutics will best neutralize these viral mutations before they can develop into global epidemics (Dickerson et al. 2007). This is phage-based method that allows rapid analysis of molecules that perturb the binding of proteins to their ligands. Because the system can amplify by replication, single-molecule sensitivity can be achieved.

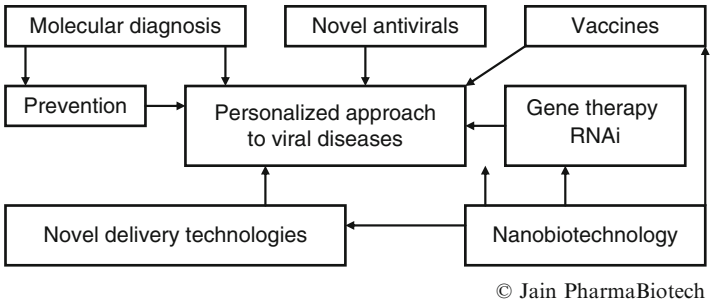


**Fig. 11.1** An integrated approach to viral diseases

When combinatorial protein or small-molecule libraries are studied, large numbers of binding events can be analyzed simultaneously. Such libraries may be used in a sequential phage escape format, where cycles of phage binding and release of mutants are driven by antibodies or small molecules and the difficulty of escape increases at each cycle. When viral systems are studied, a checkmate analysis allows experimental evaluation of the evolutionary contest between viruses and the immune system and may predict which antibodies and small-molecule ligands should be generated in anticipation of viral mutations before these mutations create viral epidemics. The result is a detailed chemical map of the trajectories of viral escape and antibody response. This enables scientists to explore all the possible routes that a virus might take to escape an immune response or small molecule therapeutics. Because this approach is both simple and inexpensive, it is within reach of almost any biomedical laboratory in the world.

Although immune mechanisms are involved in virus infection, there are no significant immune modulators available. Understanding how the viruses manipulate the host immune system may provide some clues to better therapies, both vaccines and antiviral therapeutics. MAbs are in development for some viral diseases and are promising but there are only a couple of significant products, one of which is in market and the other in late stage clinical trials. Among the novel technologies such as gene therapy, antisense and RNAi, the main problems have been delivery and although there are some clinical trials, there is no prospect of a curative viral therapy in the next few years. Personalized therapy approaches are being applied to improve antiviral therapeutics. HIV was the first viral diseases where molecular diagnostic was used to guide treatment.

## *Personalized Approach to Management of HIV*

HIV belongs to a large family of RNA lentiviruses. The result of HIV infection is destruction of the immune system leading to onset of the acquired immunodeficiency syndrome (AIDS). The AIDS epidemic has already resulted in the deaths of over half

its victims. Although long-term survival has increased by aggressive therapy with combination of antiviral agents, all HIV-infected persons are at risk for illness and death from opportunistic infections and neoplastic complications as a consequence of the inevitable manifestations of AIDS.

There are two variable factors in HIV/AIDS: how people respond to the HIV and how HIV responds to drugs. These should be taken into consideration. Personalized management of HIV/AIDS means use of the most rational and effective approach in which one method could be effective in several variants of the disease.

### Decoding the Structure of an Entire HIV Genome

HIV, like the viruses that cause influenza, hepatitis C and polio, carries its genetic information as single-stranded RNA rather than double-stranded DNA. The HIV RNA genome is very large, composed of two strands of nearly 10,000 nucleotides (building blocks) each. The information encoded in DNA is almost entirely in the sequence of its nucleotides. But the information encoded in RNA is more complex; RNA is able to fold into intricate patterns and structures. These structures are created when the ribbon-like RNA genome folds back on it to make 3D objects.

The structure of an entire HIV-1 genome at single nucleotide resolution using SHAPE, a high-throughput RNA analysis technology, has been published (Watts et al. 2009). The genome encodes protein structure at two levels. In addition to the correspondence between RNA and protein primary sequences, a correlation exists between high levels of RNA structure and sequences that encode inter-domain loops in HIV proteins. This correlation suggests that RNA structure modulates ribosome elongation to promote native protein folding. Some simple genome elements previously shown to be important, including the ribosomal gag-pol frameshift stem-loop, are components of larger RNA motifs. The authors also identified organizational principles for unstructured RNA regions, including splice site acceptors and hypervariable regions. These results emphasize that the HIV-1 genome and, potentially, many coding RNAs are punctuated by previously unrecognized regulatory motifs and that extensive RNA structure constitutes an important component of the genetic code. The study opens the door for further research, which could accelerate the development of antiviral drugs.

### Genetics of Human Susceptibility to HIV Infection

Humans are not equal in terms of susceptibility to infection to HIV, or in the rate of disease progression. This is evidenced by the identification of individuals that remain seronegative despite of multiple exposures to HIV-infected partners, and by the existence of the so called “long-term progressors”. Elite controllers’ are rare HIV-infected individuals who are able to spontaneously control HIV replication without medication, maintaining viral loads that are consistently below the limits of detection by currently available commercial assays. Studies of elite controllers may

elucidate mechanisms of HIV immune control useful in designing a vaccine. Although many elements of innate and adaptive immunity are associated with control of HIV infection, the specific mechanism(s) by which elite controllers achieve control remain undefined (Baker et al. 2009). Ongoing studies of elite controllers, including those examining host genetic polymorphisms, should facilitate the definition of an effective HIV-specific immune response and guide vaccine design.

Currently used research approaches to study individual susceptibility to HIV include:

- Analysis of the differences of susceptibility at the cellular level. This requires the characterization of the cellular permissiveness to HIV or HIV-derived lentiviruses.
- Mapping of chromosomal susceptibility loci by genome scan using linkage analysis in the in vitro setting of transduction of immortalized B cells from multigeneration families.
- Whole genome association study on a characterized population providing data on viral set point after HIV seroconversion. This is a collaborative European project supported by the Center of HIV/AIDS vaccine immunology/NIH (CHAVI).

CHAVI is a significant component to the Global HIV Vaccine Enterprise, which includes investigators from institutions across the globe with the goal of solving major problems in HIV vaccine development and design. CHAVI's initial mission is to find out what the immune system does during HIV infection – including in the rare individuals who control the infection on their own – and try to produce a vaccine to mimic those responses. Work will provide a unique description of how host genetic variation influences the early stages of HIV infection, the exposed and uninfected state, and the interindividual differences in the generation of neutralizing antibodies or in breath of cytotoxic T lymphocyte responses. The project will apply state of the art genome association studies.

The Host Genetics Core, which includes the EuroCHAVI project, will use whole genome analysis to analyze the differences in host genetic structures that indicate susceptibility to HIV-1 transmission and/or infection. EuroCHAVI aims to quickly identify common genes that affect how the body responds to HIV and the speed at which the infection progresses to AIDS. Whole genome analyses are carried out using the Infinium™ HumanHap550 Genotyping BeadChip Illumina technology. This Chip addresses more than 555,000 SNPs providing comprehensive genomic coverage across multiple populations. This large-scale genome analysis is critical for determining the role of genetic variants in a complex disease such as AIDS.

### Host-Pathogen Interactions That Regulate HIV-1 Replication

HIV-1 and HIV-2 rely upon host-encoded proteins to facilitate their replication. Scientists at the Salk Institute for Biological Studies and Burnham Institute for Medical Research combined genome-wide siRNA analyses with interrogation of human interactome databases to assemble a host-pathogen biochemical network containing 213 confirmed host cellular factors and 11 HIV-1-encoded proteins (König et al. 2008).

Protein complexes that regulate ubiquitin conjugation, proteolysis, DNA-damage response, and RNA splicing were identified as important modulators of early-stage HIV-1 infection. Additionally, over 40 new factors were shown to specifically influence the initiation and/or kinetics of HIV-1 DNA synthesis, including cytoskeletal regulatory proteins, modulators of posttranslational modification, and nucleic acid-binding proteins. Finally, 15 proteins with diverse functional roles, including nuclear transport, prostaglandin synthesis, ubiquitination, and transcription, were found to influence nuclear import or viral DNA integration. Altogether, there are 295 host cell factors that likely act in concert to facilitate the early steps of HIV-1 infection. Although it is known for a long time that HIV hijacks our cellular proteins to complete its life cycle, this study now lays out its flight plan. These findings may yield novel therapeutic targets for HIV.

### Genetic Basis of Human Body's Resistance Against HIV

The body's first response to HIV strongly influences the rate at which the virus will destroy the immune system. Shortly after infection, HIV levels rise steeply, but then the immune system and other antiviral factors produced by cells drive down the viral load and establish a "set point." The lower the set point, the longer the immune system can function effectively. These set points vary widely in individuals. Understanding why some people establish and maintain effective control of HIV-1 and others do not is a priority in the effort to develop new treatments for HIV/AIDS. Using a whole-genome association strategy, scientists have identified polymorphisms that explain nearly 15 % of the variation among individuals in viral load during the asymptomatic set point period of infection (Fellay et al. 2007). One of these is found within an endogenous retroviral element and is associated with major histocompatibility allele HLA-B*5701, while a second is located near the HLA-C gene. An additional analysis of the time to HIV disease progression implicated a third locus encoding a RNA polymerase subunit. These findings emphasize the importance of studying human genetic variation as a guide to combating infectious agents. The genetic polymorphisms could form the basis of more effective vaccines and antiviral agents against HIV. However, this study largely focused on Caucasians and the results need to be replicated in the context of a different genetic background.

### Pathogenesis of AIDS

It is important to understand the molecular mechanism by which the HIV virus infects, or integrates, healthy cells. The integration of retroviral DNA by the viral integrase into the host genome occurs via assembled pre-integration complexes. By using Fluorescence that the Integrase holds the two ends of the viral DNA together prior to integration as shown by resonance energy transfer. Once inside the cell, the two viral DNA ends are fused by the integrase to the cell's chromosome. The integrated viral DNA allows virus replication. If the two ends of the viral DNA do not

come together, infection does not take place. Millions of HIV tainted cells can be launched from a single infected cell. This technique can be used in the ongoing studies of the effects of drugs in the process of assembly and disassembly of the viral DNA integrase complexes.

The genome of HIV contains three major genes – gag, pol – and env, which code for the major structural and functional components of HIV, including envelope proteins and reverse transcriptase. The major structural components coded by env include the envelope glycoproteins, including the outer envelope glycoprotein gp120 and transmembrane glycoprotein gp41 derived from glycoprotein precursor gp160. Major components coded by the gag gene include core nucleocapsid proteins p55, p40, p24 (capsid, or "core" antigen), p17 (matrix), and p7 (nucleocapsid); the important proteins coded by pol are the enzyme proteins p66 and p51 (reverse transcriptase), p11 (protease), and p32 (integrase). Elucidating the genes influencing risk of HIV-1 infection and disease progression may help target areas for intervention, such as vaccine development.

The pathogenesis of HIV infection is a function of the virus life cycle, host cellular environment, and quantity of viruses in the infected individual. After entering the body, the viral particle is attracted to a cell with the appropriate CD4 receptor molecules where it attaches by fusion to a susceptible cell membrane or by endocytosis and then enters the cell. The probability of infection is a function of both the number of infective HIV virions in the body fluid which contacts the host as well as the number of cells available at the site of contact that have appropriate CD4 receptor.

Immune activation is a hallmark of HIV infection and a significant factor in continuous viral replication and CD4+ T cell depletion. In HIV-infected individuals, levels of circulating activation markers correlate with accelerated disease progression and shortened survival. HIV infection is critically dependent on the activated state of CD4+ T cells since quiescent T cells in blood are refractory to HIV infection. In addition, T-cell activation enhances viral transcription through activation of transcription factors, such as nuclear factor kB. HIV infection itself manipulates the activation status of infected T cells through the expression of viral proteins including the viral transactivator Tat, which potently activates HIV transcription. Tat also influences the expression of cellular genes in infected T cells. While the function of Tat in viral transcription is well studied, the molecular mechanism underlying its immunomodulatory effects is less clear.

The asymptomatic phase of HIV infection is characterized by a slow decline of peripheral blood CD4+ T cells. One potential explanation of why this decline is slow is that the low average rate of immune activation dictates the pace of a "runaway" decline of memory CD4+ T cells, in which activation drives infection, higher viral loads, increased recruitment of cells into an activated state, and further infection events. Some alternative mechanisms include the phenomenon of viral rebound, in which interruption of antiretroviral therapy causes a rapid return to pretreatment viral load and T cell counts, supporting the role of virus adaptation as a major force driving depletion.

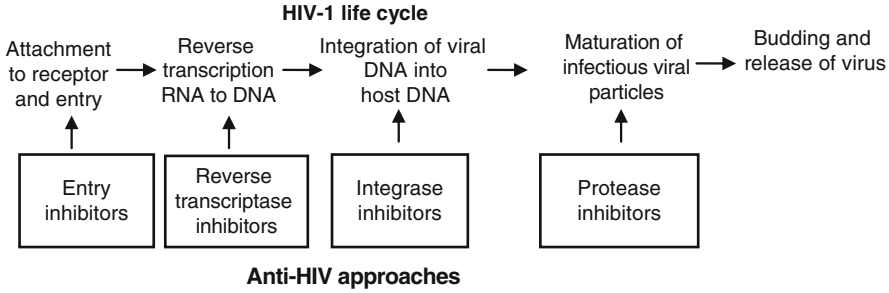


**Fig. 11.2** Mode of action of some current anti-HIV drugs

## Current Therapies of HIV/AIDS

A variety of therapies have been developed for persons infected with HIV. Bone marrow transplantation, lymphocyte transfusions, thymic transplantation, and therapeutic apheresis to remove virus-bearing cells were tried without significant success against HIV infection and are no longer employed. Current therapeutic strategies for intervening in HIV-1 disease include the following:

- Antiretroviral chemotherapeutic agents
- Treatment and prophylaxis of opportunistic infections
- Treatment of other complications including tumors

AIDS has been treated with chemotherapeutic agents that act at various key points in the life cycle of HIV (Fig. 11.2). These include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). NRTIs remain a cornerstone of current antiretroviral regimens in combinations usually with a NNRTI, a PI, or an INI.

According to International AIDS Society's guidelines for the use of antiretroviral therapy in adult HIV infection, therapy should be initiated before CD4 cell count declines to <350/μL. In patients that have 350 CD4 cells/μL or more, the decision to begin therapy should be individualized based on the presence of comorbidities, risk factors for progression to AIDS and non-AIDS diseases, and patient readiness for treatment. In addition to the prior recommendation that a high plasma viral load (e.g. >100,000 copies/mL) and rapidly declining CD4 cell count (>100/μL per year) should prompt treatment initiation, active HBV or HCV coinfection, cardiovascular disease risk, and HIV-associated nephropathy increasingly prompt earlier therapy. The initial regimen must be individualized, particularly in the presence of comorbid conditions, but usually will include efavirenz or a ritonavir-boosted protease inhibitor plus 2 nucleoside reverse transcriptase inhibitors (tenofovir/emtricitabine or abacavir/lamivudine). Treatment failure should be identified and managed promptly, with the goal of therapy, even in heavily pretreated patients, being an HIV-1 RNA level below assay detection limits.

Aim of anti-HIV drugs is to keep viremia levels or viral load as low as possible. Viral load measures how many copies of the HIV virus there are in a unit of the individual's blood. Viral load changes are often described as "log" changes. The viral load test is one of several important tools for physicians to assess the efficiency and health of a person's immune system. Current HAART anti-HIV therapies aim to destroy the viruses in circulation and reduce viremia levels. Various studies have shown that viremia levels correlate with length of survival. However, AIDS is a dynamic condition, in which the rate of creation of new virus particles is balanced by the rate of their destruction, primarily by the body's innate defenses. Viral load rises sharply if mutations occur in HIV. Secondary infection can precipitate progression to the AIDS stage, which is characterized by rapidly rising HIV viral loads (viremia), and concomitantly rapidly declining CD4+ T cells (an important component of human immune system). Eventually, the patient dies of complications related to the debilitation of immune response, often by a variety of secondary infections or even various cancers.

HAART has transformed AIDS from an inevitably fatal condition to a chronic, manageable disease in some settings. The median survival of asymptomatic HIV-infected individuals with viremia under control is ~10 years. During the asymptomatic stage, it is known that the level of the steady state viremia correlates with the future progression of the disease and the life span of the patient. In most patients, HAART therapy is usually initiated only after the CD4+ T cell count falls <350 per ul. The two important reasons for delaying the initiation of HAART are (i) patient's economic conditions, and (ii) the fear that when resistance against HAART due to mutations, there is no recourse, although changes in the drug combinations may provide "temporary" control.

A 48-week course of antiretroviral therapy in patients with primary HIV infection delayed disease progression, although not significantly longer than the duration of the treatment (SPARTAC Trial Investigators 2013). The finding that this approach altered the course of the two main markers of HIV disease progression, CD4+ count and the HIV RNA level, beyond the treatment period is an intriguing observation that requires further evaluation.

Survival varies a lot and depends on the stage HIV infection is detected and the treatment. Patients who have already developed AIDS at presentation have a high risk of mortality within 1 year, and a much worse life expectancy. Patients with newly diagnosed asymptomatic HIV infection in the HAART era may survive for >20 years after diagnosis according to current projections. These results are limited by uncertainty regarding some factors. For example, the efficacy of HAART may gradually decrease over time due to accumulation of resistance mutations. Although this may be balanced by the introduction of a more potent salvage therapy, the impaired immune functions and the adverse effects of some HAART regimens on metabolic profiles may also take their toll in the late course of the illness, and may interact synergistically rather than additively with underlying diabetes mellitus or coronary artery disease.

### Pharmacogenomics of Antiretroviral Agents

A large number of drugs with different mechanisms of action are available for the treatment of HIV. None of them is curative and there is considerable variation in the response to antiretroviral drugs among individuals. This concerns both the interindividual differences in pharmacokinetics, and in toxicity. Various research approaches are currently used are:

- Analysis of genetic variation in CYP450 and transport genes.
- Analysis of genetic variation in mitochondrial genes and lipid metabolism and transport genes to investigated the basis of metabolic and lipid disorders associated with use of specific antiretroviral agents.

A growing number of entry inhibitors are under clinical development, with some already approved. With the emergence of virus strains that are largely resistant to existing reverse transcriptase and protease inhibitors, the development of entry inhibitors comes at an opportune time. Nonetheless, because all entry inhibitors target in some manner the highly variable Env protein of HIV-1, there are likely to be challenges in their efficient application that are unique to this class of drugs. Env density, receptor expression levels, and differences in affinity and receptor presentation are all factors that could influence the clinical response to this promising class of new antiviral agents.

SensiTrop test (Pathway Diagnostics) is a molecular-based assay for co-receptor tropism that helps physicians personalize HIV therapy. It will identify the patients being treated for HIV infection that will benefit from entry inhibitor drugs. Quest Diagnostics has licensed the heteroduplex tracking technology used in SensiTrop test and is developing a validated test based on this.

### Pharmacogenetics and HIV Drug Safety

Pharmacogenetics could benefit HIV therapeutics because of the high prevalence of drug-related adverse events and the long term nature and complexity of combination therapy. There are a number of pharmacogenetic determinants of antiretroviral drug exposure, toxicity, and activity. Studies across the world have consistently demonstrated that HLA-B*5701 predicts the likelihood of hypersensitivity reactions to abacavir. As a consequence, pharmacogenetic screening for HLA-B*5701 has entered routine clinical practice and is recommended in most guidelines before starting an abacavir containing regimen. A novel HLA-B*57:01 screening test has been described, which can be easily implemented by those laboratories already involved in the detection of viral load and virus genotyping (Russo et al. 2011).

Several prospective clinical trials and cohort studies have identified a number of associations between human genetic variants, drug metabolism and toxicity. These include nevirapine hypersensitivity and hepatotoxicity, efavirenz plasma levels and

central nervous system side effects, indinavir- and atazanavir-associated hyperbilirubinemia, antiretroviral drug-associated peripheral neuropathy, lipodystrophy and hyperlipidemia, NRTI-related pancreatitis, and tenofovir-associated renal proximal tubulopathy. Thus, pharmacogenetics is expected to play an important role in HIV treatment in the near future.

### Role of Diagnostic Testing in Management of HIV

Role of diagnostic testing in management of patients with HIV infection is as follows:

- Detection of HIV-infected individuals
- Evaluation of newly-diagnosed patients
- Monitoring of therapeutic regimens
- Prognosis of disease progression (CD4 plus viral load)
- Management of drug resistance
- Prevention of adverse reactions to drugs

A number of assays have been developed to detect the presence of HIV-1 infection and quantify the level of virus in the blood of infected individuals:

- EIA tests for the detection and quantification of HIV-1 p24 antigen.
- Western blot.
- Latex agglutination.
- Radioimmunoprecipitation.
- Immunofluorescence for the detection of antibodies to HIV-1.
- Viral cultures for the isolation and semiquantification of HIV-1.

ViroSeq HIV-1 Genotyping System (Celera) has been cleared by the FDA and is CE Marked for use in detecting HIV genomic mutations that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection. ViroSeq can be used for managing therapy changes in HIV-1 infected patients whose drug regimen has failed and in HIV-1 infected individuals at initial presentation, before starting treatment. ViroSeq's high throughput processing provides an integrated system from sample preparation to the final interpretive resistance report to aid in treatment decisions. ViroSeq can be used to detect HIV-1 subtype B viral resistance in samples with a viral load ranging from 2,000 to 750,000 copies/mL and provides sequence analysis of the entire HIV-1 protease gene from codons 1–99 and two-thirds of the reverse transcriptase gene from codons 1–335.

In 2011 ViiV Healthcare, which was formed by GlaxoSmithKline and Pfizer in 2009 to focus on HIV medications, started a phase III trial for Celsentri/Selzentry. Called MODERN (Maraviroc Once daily with Darunavir Enhanced by Ritonavir in a Novel regimen), the trial compares Celsentri/Selzentry with Truvada (emtricitabine/tenofovir), both in combination with darunavir/ritonavir. The 96-week trial assessed a 2-drug regimen against a 3-drug regimen for treating antiretroviral-naive patients infected with CCR5-tropic HIV-1. MODERN was the first large-scale trial

that compared a genotypic test with a phenotypic test for identifying patients who may benefit from Celsentri/Selzentry. Siemens Healthcare Diagnostics (SHD) provided genotypic testing in the trial, while Monogram Biosciences (subsidiary of LabCorp) provided phenotyping testing. In 2011, SHD started a partnership to make its Trugene molecular HIV-1 test compatible with Illumina's MiSeq benchtop sequencing system and to set new standards for the use of NGS for the identification of HIV-1 and potential treatments paths. The study was terminated in 2013 following a preliminary review of the Week 48 primary efficacy data by the study's external independent Data Monitoring Committee (DMC). The DMC assessed the data as demonstrating significant differences between the treatment arms in virologic responses and failures. The DMC recommended and the Sponsor concurred that the study be terminated because of the inferior efficacy of the Maraviroc arm as compared to the comparator arm (Emtricitabine/Tenofovir).

### CD4 Counts as a Guide to Drug Therapy for AIDS

When patients are infected with HIV/AIDS, the number of circulating CD4 T-cells drops significantly. CD4 counts assist in the decisions on when to initiate and when to stop the treatment, which makes this test so important. While such testing is routine in Western countries and used repeatedly over the course of treatment to see if interventions are effective it is unavailable to many people in the developing world, especially in rural areas. A cheap test for CD4 plus T lymphocytes in the blood is in development using biosensor nanovesicles to enhance the signal.

### Role of Biomarkers in Management of HIV/AIDS

Direct detection of HIV-1 is difficult because only a small number of cells harbor the virus, a small number of proviral copies exist in each infected cell, and the viral genome has a tendency toward transcriptional dormancy. There is a need for biomarkers that can be used to monitor the course of HIV/AIDS and to assess the effect of antiretroviral therapy.

APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G; also known as CEM15, or hA3G) is a novel cellular factor of innate immunity that inhibits HIV replication in vitro by causing G to A hypermutations, and consequently reduced relative infectivity of each virus produced by infected cells. Quantification of CEM15 mRNA levels in patient samples has been used as a prognostic indicator of innate HIV/AIDS disease resistance and for predicting whether a viral infected patient will be categorized as a long term nonprogressor, which has a much slower disease progression rate (Jin et al. 2007). This also provides a method of predicting the level of CD4 cells in a patient, as well as a method of optimizing antiviral therapy in a viral infected patient and has significant implications on new development of diagnostic tools and therapeutic targets to treat viral infections.

Systems biology approaches are providing new clues into host-pathogen interactions and help our understanding of the contributions made by innate and adaptive immune defenses to prevent limit infection with HIV and limit disease progression. This will be critical for developing novel approaches to HIV prophylaxis and therapy by validating new biomarkers predictive of disease outcome and treatment efficacy (Peretz et al. 2012).

**Drug-Resistance in HIV/AIDS**

Drug-resistance is a critical factor contributing to the loss of clinical benefit of currently available HIV therapies. Accordingly, combination therapies have been used to address the rapidly evolving virus. However, there has been great concern regarding the growing resistance of HIV-1 strains to current therapies as multidrug resistance to protease inhibitors is becoming more common.

HIV protease inhibitor (PI) therapy results in the rapid selection of drug resistant viral variants harboring one or two substitutions in the viral protease. To combat PI resistance development, two approaches have been developed; (1) to increase the level of PI in the plasma of the patient; and (2) to develop novel PI with high potency against the known PI-resistant HIV protease variants. Both approaches share the requirement for a considerable increase in the number of protease mutations to lead to clinical resistance, thereby increasing the genetic barrier. Nevertheless, HIV can use an alternative mechanism to become resistant to PI by changing the substrate instead of the protease. Increased polyprotein processing due to mutations in the natural substrate of the HIV protease might be a new mechanism by which HIV can become resistant to PIs. This enables HIV to develop PI resistance without the need for multiple changes in its protease and thus avoids the high genetic barrier to resistance that new PIs provide.

The level of resistance to antiretroviral drugs differs among HIV variants. There is limited knowledge of resistance mutations in non-B subtypes of HIV type 1 (HIV-1) and their clinical relevance, despite the fact that >90 % of patients with HIV-1 infection worldwide have non-subtype B variants of HIV-1 (Wainberg et al. 2011). Most reports on drug resistance deal with subtype B infections in developed countries.

The development of resistance is driving research to identify new drugs targeting novel steps in the HIV-1 replication cycle. Progress has been made in developing drugs targeting HIV-1 entry, integration and maturation. The addition of new drugs to the existing therapeutic arsenal will improve treatment options and clinical prospects particularly for those patients failing current drug regimens based primarily on combinations of reverse transcriptase and protease inhibitors. Despite the negative impact of drug resistance in the clinic, understanding resistance mechanisms provides a powerful tool to aid the discovery and development of new HIV-1 therapies.

The large number of therapy options makes it difficult to select an optimal therapy, particularly in patients that develop resistance to some drugs. Computer-based therapy selection, which assesses the level of viral resistance against drugs, has become a mainstay for HIV patients as shown in Fig. 11.3.

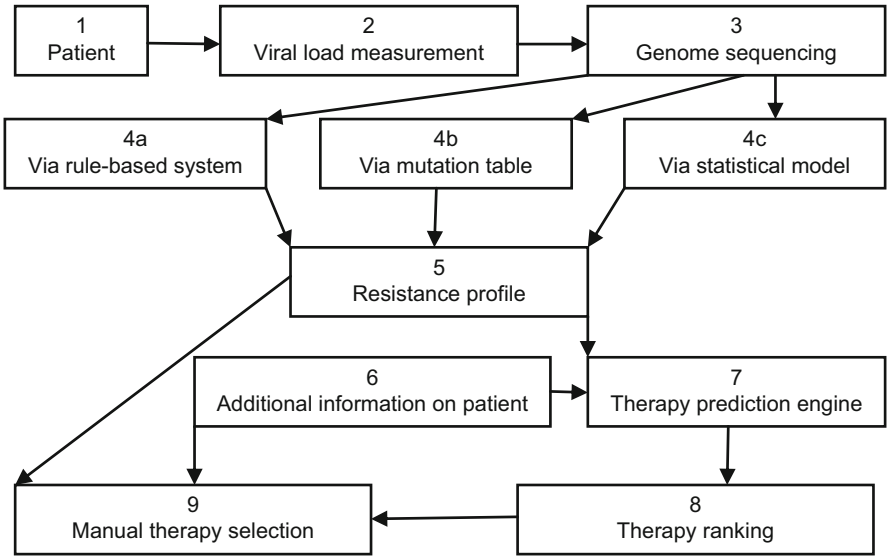


**Fig. 11.3** Workflow of genotypic resistance analysis for personalized HIV therapy. Workflow of current-day genotypic resistance analysis. The process begins by detecting the viral load (*2*) in a patient (*1*). In the case of anticipated therapy change the viral genome is sequenced from the patient's blood serum (*3*). Interpretations of the viral genome sequence is effected either manually using a mutation table (*4a*), or via a rules-based system (*4b*), or with a statistical model derived from clinical resistance data (*4c*). The interpretation results in a resistance profile (*5*) that is qualitative in the first two cases and quantitative when using statistical models. The physician uses this profile to select a therapy (*9*). In doing so, additional information on the patient is also taken into account (patient history, habits, drug side effects, etc. (*6*). Therapy prediction engines (*7*) can assist this process by a quantitative analysis that yields a list of therapies ranked by their likelihood of success (*8*) (Source: Lengauer et al. 2014)

### Sequencing for Detecting Mutations to Personalize HIV Therapy

According to work being done at the David Bioinformatics Lab of the US National Institute of Allergy and Infectious Diseases, long-read sequencing can enable researchers to not only detect what drug-resistance mutations are present in someone with HIV, but also whether those mutations are present in one strain or spread across multiple strains of the virus circulating in that patient. Sanger sequencing has been used to detect drug resistance mutations in HIV, but its ability to perceive minor mutations is limited. Although NGS improves upon that sensitivity to detect rare mutations, newer long-read approaches can detect both mutations and quasispecies of the HIV virus. This approach has enabled detection of rare mutations in HIV. Additionally, by examining patient samples taken at different time points, it is also possible to determine how previously rare mutations became more common.

Most of resistance mutations crop up in the stretch of HIV genome that houses its protease and reverse transcriptase genes. To detect drug resistance mutations, this 1.4 kilobase region is amplified using PCR and genotyped using the Sanger-based

TruGene kit (Siemens), which is run on Illumina's MiSeq. Other NGS approaches like Roche/454, and Life Technologies' Ion PGM also have a high sensitivity for detecting these minor mutations. However, short-read approaches lose the linkage relationship between the mutations although they can detect multiple mutations, but not whether they were all in one strain or housed among a few strains circulating in the patient.

A long-read approach, such as the Pacific Biosciences platform, could give both the frequency and phasing information because its read length covers the full length of the PCR product. Reads longer than 10 kilobases are common, and efforts are being made to further increase the average read length.

In a comparative approach, the Sanger-based approach was unable to detect rare mutations <20 % frequency; the NGS approaches were sensitive down to about 1 %; and the PacBio approach could detect them down to nearly 0.1 %. Knowing which mutations are present and their phasing information can help clinicians decide upon a drug treatment regimen for the patient. Different drugs might be needed to target a virus strain with two mutations as compared to two strains with one mutation each.

### PhenoSense® to Test HIV Drug Resistance

PhenoSense® GT (Monogram Biosciences/LabCorp) is a resistance test that combines three tests – PhenoSense® HIV, GeneSeq® HIV and Replication Capacity (RC) – to make up one complete picture of drug resistance. The combination test is performed from the same blood sample and the results are in one report. PhenoSense® GT looks at an individual's HIV using two different methods, so that the most effective treatment can be selected. It is a direct measure of the virus' ability to replicate in given concentrations of antiviral compounds as measured by the phenotypic portion of PhenoSense® GT. The patient virus is also sequenced, with the genotypic data provided alongside the susceptibility results. Finally, it measures the ability of the viral protease and reverse transcriptase to drive replication – known as replication capacity, one component of viral fitness. PhenoSense® GT offers consistent results because both phenotypic and genotypic results come from the same blood sample, and some of the discrepancies between phenotypic measurements and genotypic predictions are resolved as part of the assay.

### Gene Therapy Strategies in HIV/AIDS

The possibility of treating AIDS by gene therapy stems from the consideration that HIV infection, like any other viral infection, is a genetic disorder resulting from acquisition of new genetic material via an infectious process. Integration of the viral genes into chromosomal DNA becomes a stable, inheritable feature of the cell genome. A human anti-HIV antibody gene can be transduced into human

lymphocytes by using an AAV vector system. The infection of several primary HIV-1 patient isolates can be effectively blocked in the transduced lymphocytes by combined intra – and extra – cellular binding activities of the neutralizing antibody. This strategy is useful for the treatment of HIV-1 infected patients.

**Personalized Vaccine for HIV**

Several vaccines are in development for HIV/AIDS. The first clinical trial of individualized treatment for HIV/AIDS patients using AGS-004 based on Arcelis technology (Argos Therapeutics) has been successfully completed in Canada. AGS-004 is a vaccine made for the individual patient instead of an off-the-rack treatment. The new therapy is based on dendritic cells which are removed from each HIV-infected patient and subsequently multiplied in vitro. By priming the immune system, as with a vaccine, to fight the specific strain of HIV/AIDS infecting a given patient, this would be a more effective weapon against the virus than the antiretroviral cocktails currently in use. Not only were there few reported side-effects from the AGS-004, but the investigators also measured increased levels of CD8-lymphocytes in the patients thus confirming that the intervention was targeted and controlled (Routy et al. 2010). Phase II of the clinical trial is testing the therapy's efficacy on its own at eight different sites in Canada.

## *Personalized Treatment of Hepatitis B*

Monitoring of HBV viral load is the most widely used method in assessing liver disease severity, predicting development of cirrhosis and hepatocellular carcinoma, deciding about initiation of antiviral therapy, assessing treatment response as well as early detection of emergence of drug resistance. Clinical outcome and efficacy of antiviral treatment might vary with HBV genotype. The importance of covalently closed circular DNA is also becoming apparent in this regard. Further studies on the development of newer molecular methods for a better management of chronic hepatitis B will minimize morbidity (Chakravarty 2012).

Treatment of chronic hepatitis B with interferon (IFN)-α results in sustained loss of virus replication in as many as 50 % of patients. The immunologic disposition of the host and genetic factors of the virus itself are probably the main determinants for an IFN response. There is indeed increasing evidence for the existence of IFN-sensitivity determining regions in the genome of hepatitis viruses. In this setting, known predictive parameters for an IFN response, such as hepatitis B virus (HBV) DNA titers, alanine aminotransferase levels, the degree of liver inflammation, and disease duration, must be considered merely as surrogate markers. Mutations in the HBV gene also influence response to IFN. With the increasing progress in nucleic acid technologies, investigation of viral genetic biomarkers may be integrated in clinical diagnostic routine.

SeqHepB program (Evivar Medical) is a unique viral genomics sequence analysis program that is linked to a HBV genomic database. It is offered as a web-based decision support tool to assist physicians to optimize and individualize the treatment schedule of patients with chronic hepatitis B. The system can be used to identify HBV mutations present in the patient and to determine resistance levels to the various available medications.

A HBV Sequencing test (Abbott Molecular) has received CE Mark for identifying genomic sequences of HBV to guide or monitor therapy, and predict or discover drug resistance. It is not intended for screening blood, plasma, or tissue donors for HBV or as a diagnostic test to confirm HBV infection. ViroSeq® HBV Assay (Celera) is in development for detection of resistance to antiviral treatment in hepatitis B.

## *Personalized Treatment of Hepatitis C*

Hepatitis C is the most common blood-borne viral infection in the US and it is one of the main causes of chronic liver disease. It is estimated that at least 4 million persons in the US and 170 million persons world-wide are infected with HCV. The complications of chronic hepatitis C, including cirrhosis and hepatocellular carcinoma, are expected to increase dramatically world-wide over the next 10–20 years. Immunomodulatory/anti-viral therapy, employing IFN-α, both alone and in combination with ribavirin, affords the only effective treatment for hepatitis C. Accurate early prediction of response to IFN therapy may decrease or eliminate unnecessary or ineffective treatment, permit greater flexibility in tailoring therapy on an individual basis, and enhance the cost-effectiveness of treatment. Liver biopsy provides valuable information about the baseline severity and subsequent progression of hepatitis C. Severe fibrosis or cirrhosis on the pre-treatment liver biopsy is associated with decreased response rates.

### Genotype and Response to Treatment for Hepatitis C

The current standard of care for HCV is PegIFN-α in combination with ribavirin. The treatment regimen lasts for 6–12 m and can lead to a permanent cure in some patients, depending upon the genotype of HCV infection and other factors not well understood. Response rates to currently approved therapies also vary by genotype, with genotype 2 and 3 patients enjoying a 76 % response rate to the current standard of care while patients with genotype 1a and 1b have only a 46 % response to the current standard of care. Unfortunately HCV genotype 1 accounts for 60 % of global infections and is the dominant strain in the US, Japan and Western Europe. In a randomized study of patients infected with HCV genotype 1, the rates of sustained virologic response and tolerability did not differ significantly between the 2 available peginterferon-ribavirin regimens or between the 2 doses of PEG-IFN α-2b (McHutchison et al. 2009).

Although the recommended treatment for chronic HCV infection involves PegIFN-α-2b or PegIFN-α-2a combined with ribavirin (RBV), the therapy cures only ~40 % of those with HCV, and the response is even lower in African-American populations. In addition to limited efficacy, treatment is often poorly tolerated because of side effects that prevent some patients from completing therapy. For these reasons, identification of a biomarker of response to treatment is a high priority.

Growing body of evidence shows that ethnicity plays a pivotal role in how patients respond to treatment for HCV. A multicenter, open-label, nonrandomized, prospective study (LATINO Study) has evaluated the effect of Latino ethnic background on the response to treatment with peginterferon alfa-2a (Pegasus®) and ribavirin in patients infected with HCV genotype 1 who had not been treated previously (Rodriguez-Torres et al. 2009). The primary end point was a sustained virologic response. The rate of sustained virologic response was higher among non-Latino whites than among Latinos and absence of HCV RNA in serum was more frequent in non-Latino whites throughout the treatment period. Poor response rate across Hispanics of all nationalities indicates that strategies to improve the sustained virologic response in Latinos are needed. A genetic polymorphism near the IL28B gene, encoding IFN-lambda-3, has been reported to be associated with an approximately twofold change in response to treatment, both among patients of European ancestry and African-Americans (Ge et al. 2009). Almost 80 % of those with the favorable response genotype eradicated the virus, while only about 30 % with the less favorable response genotype did so. Because the genotype leading to better response is in substantially greater frequency in European than African populations, this genetic polymorphism also explains approximately half of the difference in response rates between African-Americans and patients of European ancestry. On the other hand, among African Americans who did carry the CC genotype, treatment response was 53.3 %—higher than the 33.3 % treatment response observed among individuals of European descent who had the TT genotype. The favorable C allele also tended to be found less frequently in those with chronic HCV infections, suggesting a role in overall viral clearance. Unexpectedly though, the authors reported that the C alleles actually appeared to be linked to higher rather than lower baseline viral loads. More research is needed to determine whether the newly identified SNP is a biomarker for other important genetic changes or whether the change itself directly influences treatment outcomes.

The measurement of viral RNA levels and genotyping may be used to optimize individual patient treatment. Genotype non-1 and a low viral load are the most significant pre-treatment indicators of sustained virologic response. The most reliable predictor of a poor virologic response is continued seropositivity for viral RNA during therapy.

The genomic sequences of independent HCV isolates differ by ~10 %, and to study the effects of this variation on the response to therapy, amino acid covariance within the full viral coding region of pretherapy HCV sequences were analyzed from participants in the Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) clinical study (Aurora et al. 2009). Covarying positions were common and linked together into networks that differed by response to therapy. There were

threefold more hydrophobic amino acid pairs in HCV from nonresponding patients, and these hydrophobic interactions were predicted to contribute to failure of therapy by stabilizing viral protein complexes. Using this analysis to detect patterns within the networks, the authors could predict the outcome of therapy with >95 % coverage and 100 % accuracy, raising the possibility of a prognostic test to reduce therapeutic failures. Furthermore, the hub positions in the networks are attractive antiviral targets to suppress evolution of resistant variants. Finally, covariance network analysis could be applicable to any virus with sufficient genetic variation, including most human RNA viruses.

### Drug Resistance in Hepatitis C

Genelyzer (Toshiba Hokuto Electronics), an electrochemical DNA chip, has been used to detect resistance to treatment in patients with hepatitis C. Lab21 has patents in the area of HCV drug resistance genotyping. This intellectual property covers the analysis of genomic sequence variation in the viral serine protease gene, NS3. This enzyme has an important role in HCV replication and is one of the key areas of attack for the pharmaceutical industry. The first HCV small molecule drugs are likely to be licensed and include drugs which inhibit the activity of NS3 (telaprevir and bocepravir). Unfortunately HCV, similarly to HIV, is likely to select for resistant variants against these drugs, so it will be important to monitor patients for resistance. Lab21 is developing proprietary new assays to monitor the emergence of these genotypic variants.

### Role of Sequencing in Personalized Management of HCV

A sequencing approach to identify DNA variants can predict failure to respond to hepatitis C therapy and help to optimize treatment options for many hepatitis C patients. GWAS to identify genetic factors underlying the lack of viral clearance in most patients revealed that SNPs in the IL28B gene region can predict non-response to treatment. A high-throughput "massively parallel sequencing" approach followed by individual genotyping has been used to identify new, highly sensitive genetic predictors of drug response (Smith et al. 2011). DNA samples from responders or non-responders were pooled, so that many patients could be screened simultaneously and cost-effectively for common mutations. Compared with previous results, the genetic variants identified through this analysis were shown to predict failure to respond with high sensitivity and specificity. By predicting which patients are unlikely to respond to the standard treatment, clinicians would be able to make an informed choice about which patients should be offered newly emerging therapies. These results are promising for the personalized management of hepatitis C.

Roche Diagnostics is partnering with three Spanish entities, including two research institutes and the software developer Advance Biological Laboratories

Therapy Edge Spain to develop personalized antiviral treatment strategies for patients with chronic hepatitis C or B. Other partners include the Vall d'Hebron Institute of Research and the Networking Biomedical Research Centre in Liver and Digestive Diseases, which is comprised of eight research groups. Roche will use its 454 sequencing systems and bioinformatics analysis, coupled with other genetic and molecular analysis techniques, to apply massively parallel sequencing in developing personalized antiviral treatments for chronic sufferers. HCV and HBV exhibit great variability; a person infected with one of these viruses presents a complex population of variants comprising a structure known as 'quasi-species'. The identification of these variants may be crucial for avoiding the selection of variants resistant to the new antiviral therapies. Using 454 sequencing makes it possible to create a comprehensive profile of the complex viral populations that circulate in individuals in order to identify the quasi-species that are resistant to existing antiviral treatments.

ViroSeq® HCV Assay (Celera), sequence-based test, is in development for detection of resistance to HCV therapy. To effectively manage patient treatment decisions as new therapeutics become available, HCV resistance testing will become standard of care.

## Personalized Management of Fungal Infections

Treatment or prophylaxis of invasive fungal infection in recipients of hematopoietic stem cell transplant (HSCT) may require management of coexistent malnutrition, organ dysfunction and graft versus host disease, all of which create added potential for inter – and intra-patient variations in drug metabolism as well as drug interactions. Polymorphism is common in genes encoding pathway components of antifungal drug metabolism such as enzymes (cytochrome P450 (CYP450), glutathione S-transferase, N-acetyltransferase and uridine 5′-diphosphoglucuronosyltransferase), uptake transporters (organic cationic transporter, novel organic cationic transporter, organic anion transporter protein, organic anion transport, and peptide tranporter) and efflux transporters (breast cancer resistance protein, bile sale export pump, multidrug and toxin extrusion type transporter, multidrug resistance protein, permeability glycoprotein, and urate transporter). Specific polymorphisms may be generalized throughout a population or largely confined to ethnic groups. CYP450 enzymes, especially 2C9 and 2C19, exhibit extensive polymorphism and are central to the metabolism of azole antifungals and their interactions with other drugs including calcineurin inhibitors, cytotoxics and benzodiazepines. Polymorphism may ultimately affect drug efficacy: CYP2C19 variation leads to a fivefold variation in voriconazole levels between individuals. In the future, routine provision of pharmacogenomic data for new drugs together with accumulating knowledge about established agents will challenge physicians to assimilate and apply that information in drug prescribing (Ashbee and Gilleece 2011).

## Personalized Management of Malaria

Worldwide there are ~500 million new cases of malaria per year. Malaria is caused by a protozoan infection of red blood cells with one of four species of the genus plasmodium: *Plasmodium falciparum, P. vivax, P. ovale, or P. malariae* are responsible for up to 2.7 million deaths yearly. Chloroquine, developed in the 1940s, was the mainstay of prevention and treatment at one time. Development of resistance to this drug has limited the efficacy in most parts of the world. There are few effective treatments available. Verpamil, when given in combination with chlorquine, reverses the drug resistance partially. This parallels the ability of verapamil to inhibit drug resistance in cancer cells. Malarone (GlaxoSmithKline), a combination of atovaquone and proguanil), is approved as a treatment of malaria resistant to cholorquine. The main focus of research now is development of therapies based on genomic knowledge of the *P. falciparum*.

### *Genomics of Malaria*

In the malaria genome sequencing project, DNA sequences of chromosomes 2, 3, 10, 11 and 14 are already determined with several others nearing completion. The US Naval Medical Research Center (Bethesda, MD) and the NIH are major backers of these efforts. The Stanford University (Palo Alto, CA) and The Institute of Genome Research (Rockville, MD) serve as the two principal US sequencing centers, while the Sanger Center (Cambridge, UK) is the main site in the UK for sequencing the DNA of several *P. falciparum* chromosomes.

With some *P. falciparum* chromosomal sequences completed and others nearing completion, considerable effort is going into understanding gene compositions and expression patterns of the parasite. The aim is to build a comprehensive picture of the parasite's multi-staged, genetically determined life style in the search for vulnerable points where drugs are most likely to block its host-debilitating actions. The genomic information can be used to develop effective malaria vaccines, each of which is aimed at a different life stage of the parasite. The term "vaccinomics" has been used to describe the comprehensive, genomics-based effort to develop a working vaccine. The gene sequence is providing many new drug targets. For instance, the genome encodes several genes specifying ABC-transporter proteins that they are implicated in drug resistance.

There are associations between chloroquine resistance and mutations in mdr-like gene (pfmdr 1) on chromosome 5 that encodes a protein Pgh 1 located in the lysosomal membrane of the parasite. A mutation of pfcrt – a gene on chromosome 7 that encodes a transmembrane protein pfCRT in the lysosomal membrane – is required to confer basic resistance before a mutation in pfmdr 1 can increase the resistance. Screening for pfcrt mutations in populations at risk can be used to monitor for resistance and this knowledge has major implications for the design of rational new drugs for malaria.

Through rapid genetic adaptation and natural selection, the *P. falciparum* parasite, the cause of the most serious form of malaria, is able to develop resistance to antimalarial drugs, defeating present efforts to control it. GWAS provide a critical hypothesis-generating tool for understanding how this occurs. However, in *P. falciparum*, the limited amount of linkage disequilibrium hinders the power of traditional array-based GWAS. Feasibility and power improvements gained by using WGS for association studies has been demonstrated (Park et al. 2012). The authors analyzed data from 45 Senegalese parasites and identified genetic changes associated with the parasites' in vitro response to 12 different antimalarials. To further increase statistical power, they adapted a common test for natural selection, XP-EHH (cross-population extended haplotype homozygosity), and used it to identify genomic regions associated with resistance to drugs. Using this sequence-based approach and the combination of association and selection-based tests, they detected several loci associated with drug resistance. These loci included the previously known signals at *pfcrt, dhfr*, and *pfmdr1*, as well as many genes not previously implicated in drug-resistance roles, including genes in the ubiquitination pathway. The success of the analysis presented in this study and demonstrated shortcomings of array-based approaches support a complete transition to sequence-based GWAS for small, low linkage-disequilibrium genomes like that of *P. falciparum*.

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# Chapter 12
# Personalized Management of Neurological Disorders

## Introduction

The general principles of personalized medicine apply to neurological disorders and this may be referred to as personalized neurology (Jain 2005). Role of omics in the development of personalized neurology will be described in the following sections. Neurogenomics is an important basis but "genomic neurology" is not an appropriate synonym for personalized neurology in the same way as genomic medicine is not a synonym for personalized medicine as pointed out in Chap. 1. Combination of genomic, proteomic, and metabolomic approaches may yield novel insights into molecular mechanisms of disease pathophysiology, which could then be integrated and translated into clinical neurology (Gotovac et al. 2014). Personalized medicine existed long before the advent of genomic age and non-genomic factors are also taken into consideration in personalizing therapy.

## Neurogenomics

Approximately 80 % of the ~19,000 human genes are expressed in the brain, and 5,000 of these exclusively in the brain and not in other organs. Neurogenomics is the study of genes in the nervous system. Of particular interest in neurology are the genes involved in neurologic disorders. In a broad sense, neurogenomics is the study of how the genome as a whole contributes to the evolution, development, structure, and function of the nervous system. The closely related term "neurogenetics" deals with the role of genetics in development and function of the nervous system as well as investigation and management of genetic disorders of the nervous system. Neurogenomics has applications in basic research, pharmaceutical industry, and in the management of neurological disorders. Many of the methods used in neurogenomics are the same as those used for genomics in general and are described in another publication by the author (Jain 2015c). Sequencing is the most important activity in this area. Relationships of neurogenomics with other omics are shown in Fig. 12.1.

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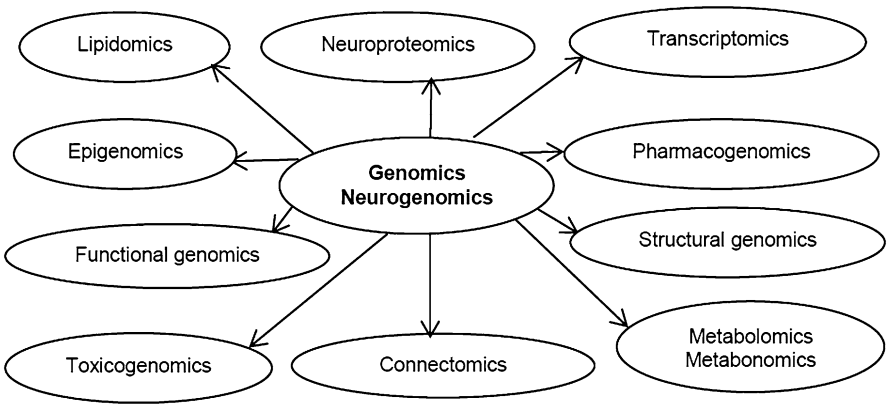


**Fig. 12.1** Relationships of neurogenomics with other omics

## *Applications of Neurogenomics in Neurological Disorders*

Many neurological conditions are caused by immensely heterogeneous gene mutations. Role of genetic factors in the etiology of complex diseases remains largely unresolved. Using genome-wide associations in millions of patient medical records, a study demonstrated that common variants associated with complex diseases are enriched in the genes indicated by the "Mendelian code" – a phenotypic code that links each complex disorder to a unique collection of Mendelian loci (Blair et al. 2013). The study identified widespread comorbidity between Mendelian-Mendelian and Mendelian-complex disease pairs.

Pathomechanism of many neurological and psychiatric disorders is poorly understood and genomic studies will not only contribute to better understanding but also improve molecular diagnostics. The current diagnostic process is often long and complex with most patients undergoing multiple invasive and costly investigations without ever reaching a conclusive diagnosis. The advent of massively parallel, NGS promises to revolutionize genetic testing and shorten the diagnostic process for many of these patients.

Genetic disorders can involve multiple systems and with predominant involvement of the nervous system, they are referred to as neurogenetic disorders. Some of the disorders described in the following sections have a significant neurogenetic component. Most of the genetic disorders are caused by point mutations. Deletions are less frequent and may be overlooked by DNA mapping. It is difficult to find the location of a gene buried in the tangle of chromosomal DNA in the nucleus. WGS may facilitate identification of alleles that cause disease. However, even in cases with simple patterns of inheritance, the relationship between disease phenotypes and their corresponding genetic changes can be complicated. Comprehensive diagnostic assays must therefore identify all possible DNA changes in each haplotype and determine

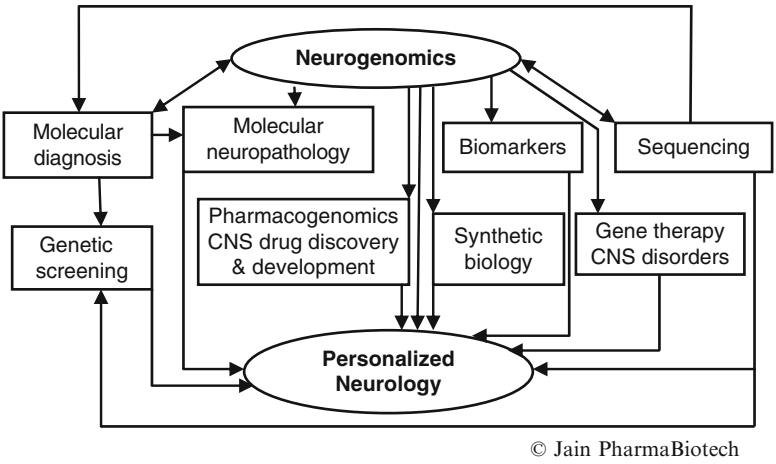


**Fig. 12.2** Role of neurogenomics in the development of personalized neurology

**Table 12.1** Applications of genomics in neurology

| |
|---|
| Basic neurosciences |
| Molecular neuropathology |
| Study of genes for neurologic disorders |
| Genomic studies of nonhuman organisms relevant to neurology |
| Creation of transgenic models of neurologic disorders |
| Brain mapping |
| Diagnosis of neurologic disorders |
| Redefinition and reclassification of disease |
| Molecular diagnostics |
| Biomarkers |
| Integration of diagnostics and therapeutics |
| Neurotherapeutics |
| Molecular neuropharmacology |
| Drug discovery and development |
| Gene therapy |
| Personalized neurology |



which are responsible for the underlying disorder. The high number of rare, heterogeneous mutations present in all humans and the paucity of known functional variants in >90 % of annotated genes make this challenge particularly difficult.

Role of neurogenomics in the development of personalized neurology is shown schematically in Fig. 12.2 and various applications of neurogenomics are listed in Table 12.1. Role of neurogenomics will be described in the following sections along with the personalized management of various disorders. Many other factors besides genomics are taken into consideration in tailoring the treatment to an individual patient.

## *Impact of Neurogenomics on the Development of Personalized Neurology*

Genomics is improving our understanding of neurologic diseases. This will be an important basis for the development of rational therapies in integrated healthcare of the future. Genomics will have the following impact on healthcare:

- Increase in the range of diseases that can be treated with drugs.
- Increase in the precision and effectiveness of drugs. If a patient can be diagnosed in terms of DNA mutations, alleles, or polymorphisms pertaining to a specific disease, their response to treatment can be vastly improved.
- An increase in the ability to anticipate diseases rather than just reacting to them. This may enable the institution of preventive measures.
- Development of more effective drugs may lead to a trend for treatment with drugs rather than surgery.

With the sequencing of the genome and genetic redefinition of neurologic diseases, pathomechanism will be better understood and will facilitate early detection by molecular methods and effective strategies for management. Availability of low-cost genomic sequencing will expand the use of genomic information in the practice of neurology. Drugs will be targeted better to diseases in particular patients based on genotype information. Toxicity will be predictable in most cases prior to drug administration. These will be significant contributions to personalized neurology.

## *Epigenomics/Epigenetics*

The epigenome is a record of the chemical changes to the DNA and histone proteins of an organism, which can be inherited by an organism's offspring. The epigenome is involved in regulation of gene expression, development, and tissue differentiation. Unlike the underlying the genome which is largely static within an individual, the epigenome can be altered by environmental conditions. Changes in the epigenome can result in changes in function of the genome.

Epigenetics refers to the study of changes in the regulation of gene activity and expression that are not dependent on gene DNA sequence. Whereas epigenetics often refers to the study of single genes or sets of genes, epigenomics refers to more global analyses of epigenetic changes across the entire genome.

Neurological disorder are not only associated with genomic mutations and transcriptomic dysregulations, but with changes in the epigenome. Among the various types of epigenomic modifications, DNA methylation, histone modifications and expression levels of microRNAs (miRNAs) have been the most widely studied. DNA methylation is implicated in the development of human brain as well as plasticity underlying learning and memory. Widespread reconfiguration occurs in the methylome and the conserved non-CG methylation accumulates in the neuronal genome during development (Lister et al. 2013).

Targeting the complete mitochondrial exome provides a greater potential to identify rare variants that disrupt normal mitochondrial function, enabling an exact diagnosis in a large proportion of patients that remain undiagnosed by other methods. Over 95 % of the target bases can be sequenced to an average coverage of 400×, providing highly accurate and sensitive results.

## Neuroproteomics

The role of proteomics in personalized medicine has been described in Chap. 6. Neuroproteomics is the term used for application of proteomics to the study of the nervous system and its disorders with the aim of developing diagnostics and therapeutics (Jain 2002). Proteomics tools offer new ways to analyze networks of proteins that control important neurobiological phenomena. Neuroproteomics, combined with bioinformatics, can be used to study the organization of functional protein networks and molecular structures that underlie physiological, anatomical, and behavioral processes (Bayés and Grant 2009).

### *Applications of Neuroproteomics for Study of the Nervous System*

Proteomics technologies are used for the study of neurotransmitters and neuronal receptors. A brief description of these is as follows (Jain 2013):

**Neurotransmitters** Capillary electrophoresis has been combined with highly sensitive micro-electrospray-tandem mass spectrometry to simultaneously detect classical small molecule neurotransmitters as well as neuropeptides from discrete regions of the brain. Endogenous glutamate, gamma-aminobutyric acid, acetylcholine, and dopamine as well as the neuropeptides methionine-enkephalin and substance P 1–7 could be detected in the striatum using only a minute amount of brain tissue. A disease-specific quantitative analysis of a specific neurotransmitter of interest may require stabilization by inactivation of the degrading enzymes present in the CSF.

**N-methyl-D-aspartate (NMDA) Receptors** This is one of the ~100 neuronal receptors known in humans. Proteomics technologies, such as mass spectrometry, can be used to characterize multiprotein complexes of NMDA receptors, which are encoded by activity-dependent genes.

**Neuroprotection** Use of neuroproteomics, systems biology, and bioinformatics aim to study and establish a global assessment of the entire neuronal proteome (Raad et al. 2012). Neuroproteomics aids in the understanding of molecular mechanisms of neurogenesis. Potential neuroprotective pharmacological strategies can be targeted at Rho and Rho kinases, which constitute key integral points in the pathway that is known to be disrupted in multiple neuropathologies, such as spinal cord injury and traumatic brain injury (Jain 2011).

**Regeneration and Degeneration of the Nervous System** Neuroproteomic technologies have been designed to uncover the mechanisms and molecules involved in neuronal regeneration and degeneration (Sun and Cavalli 2010). Most proteins mediating regeneration are found to be either malfunctioning or reduced in degeneration.

**Study of the Blood-Brain Barrier** Proteomic technologies can be used to study neuropathology at the blood-brain barrier (BBB). Proteomics can also be applied to facilitate drug delivery across the BBB by characterizing active efflux systems that can prevent drug access to the brain and by identifying new transporters that could be used as noninvasive drug delivery conduits.

**Use of Neuroproteomics for Study of Neurologic Disorders** Neuropathologies involving loss of neurons and disturbances of neurotransmission may result in disease-specific alterations of neuronal and CSF proteins that are suitable for proteomics analysis. CNS proteomics may identify cell types and tissues contributing to the disease phenotype. Proteomics analysis of human brain tissue has been done as an extension of the study of CSF proteins. These studies are further facilitated by the availability of 2D maps of brain-specific proteins. Role of neuroproteomics in the management of individual disorders will be described in sections dealing with these disorders.

## *CSF Tests Based on Proteomics*

CSF examination provides a practical way to conduct longitudinal molecular analyses of changes during the course of neurologic disease. Integrated and parallel analyses of neurotransmitters, neuropeptides, and fingerprints of proteins in the CSF may provide a better insight into underlying pathomechanisms. Proteomics investigations of CSF have led to the discovery and validation of biomarkers, mainly in the field of neurodegenerative disorders (Gabelle et al. 2009). Some disease-specific proteins identified in the cerebrospinal fluid of patients with neurologic disorders are shown in Table 12.2.

## *Diagnosis of Neurologic Disorders by Examination of Proteins in the Blood*

**S-100** Antibody-based tests can measure proteins in the blood. Concentrations of the S-100 protein, an acidic calcium-binding protein found in the gray matter of the brain, are elevated in serum after brain damage. Measurement of serum concentrations of S-100 is a valuable tool that can be used more easily than tests on CSF in the differential diagnosis of Creutzfeldt-Jakob disease, as significantly higher concentrations

**Table 12.2** Disease-specific proteins in CSF of patients with neurologic disorders

| Proteins | Diseases |
|---|---|
| Tau proteins | Alzheimer disease |
| | Parkinson disease |
| | Creutzfeldt-Jakob disease |
| | AIDS encephalopathy |
| | Alcohol-induced organic brain disorders |
| 14-3-3 protein | Creutzfeldt-Jakob disease |
| Dopamine-releasing protein | Parkinson disease |
| Neurofilament protein | Multiple sclerosis |
| | Progressive supranuclear palsy |
| Myelin basic protein | Multiple sclerosis |
| | Optic neuritis |
| Neuron-specific enolase | Cerebral infarction |
| | Temporal lobe epilepsy |
| S-100 protein | Cerebral infarction |
| | Traumatic central nervous system injury |
| | Temporal lobe epilepsy |
| Glial fibrillary acidic protein | Severe neurodegeneration |
| Apolipoprotein D | Alzheimer disease |
| | Traumatic brain injury |
| Apolipoprotein E | Alzheimer disease |
| Synaptosomal-associated protein | Alzheimer disease |
| Amyloid precursor proteins | Alzheimer disease |
| Group box protein-1 | Subarachnoid hemorrhage |



are found in Creutzfeldt-Jacob disease than in other diseases. However, this test does not replace brain biopsy for definitive diagnosis of Creutzfeldt-Jakob disease.

Several commercial ELISA assays are available for S-100 protein and are useful biochemical markers for the early assessment of cerebral infarction by the quantitative determination of serum S-100. Undetectable S-100 in the blood of patients with head injury can rule out brain damage, and S-100 levels correlate with the extent of brain damage in severe head injury. Peak levels of serum S-100 correlate with neurologic deficit resulting from either stroke or traumatic brain injury, and the patterns can be used to differentiate between the two conditions. Undetectable serum level of S-100 protein predicts normal intracranial findings on CT scan in patients with traumatic brain injury. Determination of S-100 protein in serum may be used to select patients for CT scanning.

Elevated serum levels of S-100 in patients with liver cirrhosis indicate early and subclinical portal-systemic encephalopathy. It seems to be a promising biochemical surrogate marker for mild cognitive impairments due to portal-systemic encephalopathy.

**Neuron-Specific Enolase** This is a glycolytic enzyme found in the neurons and neuroendocrine cells. Increased levels of neuron-specific enolase have been measured as a result of ischemic stroke in cerebral spinal fluid as well as in blood.

**Table 12.3** Neurodegenerative diseases with underlying protein abnormalities

| Disease | Proteins involved | Inclusion bodies |
|---|---|---|
| Familial encephalopathy with myoclonus | Neuroserpin | Collin body |
| Familial Parkinson disease with Lewy bodies | Alpha-synuclein | Lewy body |
| Creutzfeldt-Jakob disease | Prion protein | vCJD amyloid |
| Alzheimer disease | β-amyloid peptide | β-amyloid plaques<br>Cofilin inclusions |
| Pick disease | Tau protein | Pick body |
| Huntington disease | Soluble huntingtin | Insoluble huntingtin |
| GM1 gangliosidosis | Deficiency of β-gal | GM1-ganglioside |



Small infarcts and transient ischemic attacks elevate only neuron-specific enolase. Peak levels of both neuron-specific enolase and S-100 are statistically significant and correlate with clinical measures of stroke size and outcome.

**Myelin Basic Protein** This is localized in the myelin sheath and constitutes approximately one third of the total protein of myelin from the human brain. Various studies have indicated that myelin basic protein concentrations in plasma and serum can be used as biomarkers for brain damage and severity of stroke. The increase of myelin basic protein in cerebral infraction is most evident several days after the onset, whereas in cerebral hemorrhage, the peak increase occurs almost immediately after the onset.

**Transthyretin** This is a protein biomarker that extravasates into the blood from the CSF only if there is disruption of the CSF-blood barrier. Transthyretin can be detected in the blood by proteomics technologies and is potentially useful for the diagnosis of diseases where the CSF-blood barrier is disrupted.

## *Role of Proteomics in Neuropharmacology*

An insight into protein-based mechanisms of neurologic disorders will provide more relevant targets for drug discovery for CNS disorders. Neurodegenerative diseases with underlying protein abnormalities are shown in Table 12.3.

Neuroproteomics studies show that neurodegenerative diseases share many common molecular mechanisms, including misfolding of proteins. Further understanding of these mechanisms may lead to strategies for prevention of neurodegenerative diseases and to the development of effective drugs. Identification of neurodegeneration-associated changes in protein expression will facilitate the identification of novel biomarkers for the early detection of neurodegenerative diseases and targets for therapeutic intervention. Applications of proteomics technologies will facilitate a more comprehensive analysis of novel therapeutic strategies for CNS disorders. This will also accelerate development of specific diagnostic and prognostic disease biomarkers.

## Neurometabolomics

Role of metabolomics in personalized medicine was described in Chap. 7. Neurometabolomics is the study of metabolites in the nervous system, many of which are biomarkers of disease. Neurons in the human CNS perform a wide range of motor, sensory, regulatory, behavioral, and cognitive functions, which requires diverse neuronal as well as non-neuronal cell types. Metabolomics can help in assessing the specificity-of metabolic traits and their alterations in the brain and in fluids such as CSF and plasma. Current applications of metabolomics include discovery of potential biomarkers of aging and neurodegenerative diseases (Jové et al. 2014). Neurometabolomics will improve our understanding of the physiology as well as pathology of the nervous system and facilitate development of personalized neurology by providing biomarkers for disease monitoring as well as for targets for therapeutics.

## Diagnosis-Guided Therapies for Neurological Disorders

The trend in healthcare in the next decade will be integration of diagnostics and therapeutics, which is an important component of personalized medicine. If patients can be diagnosed in terms of DNA mutations, alleles, or polymorphisms pertaining to a specific disease, their response to treatment can be vastly improved. This will be facilitated by gene-based disease management incorporating genetic tests that predict the safety and efficacy of therapeutic products. The identification of genes that influence the penetrance and expressivity of risk would be important in determining these risk profiles. As treatment becomes more specific to the genetic cause of disease, diagnostic tools that measure the activity of targeted genes will become crucial for disease management. Eventually, prior to the prescription of therapy, a diagnostic test must confirm the existence of diseased genes and their activity levels in order to improve treatment efficiency and cut patient care cost.

## Personalized Biological Therapies for Neurological Disorders

Personalized biological therapies were described in Chap. 9. Those particularly relevant to personalized treatment of neurological disorders will briefly described here.

Use of autologous stem cells for treatment of neurological disorders or immunotherapies of brain tumors involving tumor cells taken from a patient's tumor are by definition personalized therapies that are also examples of non-genomic approaches. The patient's own somatic cells, genetically engineered to release therapeutic substances are also in clinical trials. This approach overlaps gene therapy.

Ex vivo gene therapy involves the genetic modification of the patient's cells in vitro, mostly by use of viral vectors, prior to reimplanting these cells into the tissues of the patient's body. This is a form of individualized therapy. Personalized cell/gene therapies are described under neurological disorders where applicable.

Apart from infectious diseases involving the nervous system, therapeutic vaccines are in development for malignant tumors (e.g. glioblastoma multiforme) autoimmune disorders (e.g. multiple sclerosis), and degenerative disorders (e.g. Alzheimer disease). Alzheimer disease and stroke have important inflammatory and immune components and may be amenable to treatment by antiinflammatory and immunotherapeutic approaches including vaccines. Cancer vaccination involves attempts to activate immune responses against antigens to which the immune system has already been exposed. Some of the vaccines are personalized and will be mentioned along with personalized approaches to individual disorders in the following sections.

Monoclonal antibodies (MABs) play an important role in personalized neurology both as diagnostic and therapeutic agents. Designed to bind to specific receptors, MABs can be used to guide passage of nanomedicines across the BBB to specific targets in the brain such as glioblastoma multiforme.

## Personalized Management of Alzheimer Disease

### *Introduction to Alzheimer Disease*

Alzheimer's disease (AD) is a progressive degenerative disorder of the brain that begins with memory impairment and eventually progresses to dementia, physical impairment, and death. The cause of AD is not well understood but it likely comprises several processes that lead to intrinsic neuronal cell killing. Patients develop various psychiatric and neurological signs during the course of the disease. The prevalence rates of dementia vary significantly in different countries, but range from 2.1 % to 10.5 %. AD is the most common type of dementia, accounting for 50–60 % of all cases.

### *Diagnosis of AD*

The diagnosis of AD is currently based on clinical and neuropsychological examination. There is currently no validated or approved biomarker of AD for early detection. MRI and CT scan images of hippocampus shrinkage and, later on, global brain shrinkage are used to help diagnose advanced disease. To date there is no approved blood test available that can discriminate dementia patients from healthy individuals. A combination of characteristic plaque markers tau and amyloid (Aβ) may constitute a specific and sensitive CSF marker for AD. Genetic tests exist to identify individuals with familial forms of AD who have AD-linked mutations in the presenilin gene, and those who have specific variations in the ApoE gene linked to higher

risk of developing AD. The ApoE e4 allele, a risk factor rather than a disease gene, has a positive predictive value of 94–98 % in an individual with suspicion of AD. It is useful for predicting response to certain drugs for AD.

A complex disease like AD is difficult to attack because no single approach is adequate and the development of a single universal therapy is unlikely. The mainstay of management of AD currently consists of cholinesterase inhibitors: rivastigmine, donepezil and galantamine. Numerous neuroprotective therapies are under investigation but the only one currently marketed is memantine – a non-competitive N-methyl-D-aspartate antagonist (Jain 2015a). Proteolytic processing of the amyloid precursor protein (APP) generates Aβ peptide, which is thought to be causal for the pathology and subsequent cognitive decline in AD. The reduction in levels of the potentially toxic Aβ peptide has emerged as one of the most important therapeutic goals in AD. Key targets for this goal are factors that affect the expression and processing of the βAPP.

Various isoforms of the nitric oxide (NO) producing NO synthase (NOS) are elevated in AD indicating a critical role for NO in the pathomechanism. To study the potential structural link between the increased synthesis of NO and the deposition of nitrotyrosine in AD, the expression of neuronal NOS (nNOS), induced NOS (iNOS) and endothelial NOS (eNOS) has been analyzed in AD and control brain. Aberrant expression of nNOS in cortical pyramidal cells is highly co-localized with nitrotyrosine. Furthermore, iNOS and eNOS are highly expressed in astrocytes in AD. In addition, double immunolabeling studies reveal that in these glial cells iNOS and eNOS are co-localized with nitrotyrosine. Therefore, it is suggested that increased expression of all NOS isoforms in astrocytes and neurons contributes to the synthesis of peroxynitrite which leads to generation of nitrotyrosine. In view of the wide range of isoform-specific NOS inhibitors, the determination of the most responsible isoform of NOS for the formation of peroxynitrite in AD could be of therapeutic importance in the personalized treatment of AD.

Metabolomics of AD, which amplifies changes both in the proteome and the genome, can be used to understand disease mechanisms from a systems biology perspective as a noninvasive approach to diagnose and grade AD. This could allow the assessment of new therapies during clinical trials, the identification of patients at risk to develop adverse effects during treatment and the final implementation of new tools towards a more personalized management of AD (Barba et al. 2008).

The low serum albumin level in AD is associated with a greater response to donepezil. In one study, cognition improved during the first 15 months of treatment in the low serum albumin level group, but worsened in patients with high albumin levels (Rozzini et al. 2008). This observation suggests that serum albumin level should be monitored to evaluate the clinical efficacy of cholinesterase inhibitors for the treatment of AD.

SPECT has been shown to identify a neuroanatomical predictor of the cognitive effects of donepezil treatment in patients with AD. Lower pretreatment regional cerebral blood flow levels in the right orbitofrontal cortex (OFC) predict a better improvement in the ADAS-cog score in response to donepezil therapy (Hongo et al. 2008). This effect may reflect the choline acetyltransferase activity associated with the OFC.

## *Genomic Basis of Personalized Approach to Alzheimer Disease*

Alzheimer's disease (AD) is a polygenic disorder and several genes as well as polymorphisms are being identified. These are mostly associated with membrane proteins. Their role as a risk factor and relation to certain forms of AD is reported and is under further investigation. Genomic research in AD has increased the understanding of pathomechanisms leading to neurodegeneration and dementia. Identification of rare, disease-causing mutations in amyloid precursor protein (APP), PSEN1, and PSEN2 causing early-onset familial AD, was followed by the discovery of APOE as the single most important risk factor for late-onset AD (LOAD). Later genome-wide association studies delivered several additional AD susceptibility loci that are common in the general population, but exert only very small risk effects. As a result, a large proportion of the heritability of AD continues to remain unexplained by the currently known disease genes.

The interaction of different transcription factors with the regulatory region of the ApoE gene plays an important role in the neuroinflammatory process seen in AD and is a target for developing new therapeutics for the disease. Genotype-specific responses of AD patients to a particular drug or combination of drugs have been demonstrated, although several studies examining the role of ApoE have produced conflicting results. The pharmacogenomics of AD may, in the future, contribute to optimizing drug development and therapeutics, increasing efficacy and safety, and reducing side effects.

Associations between the GAB2 gene and LOAD risk has been characterized in APOE 34 carriers by genome-wide survey of >300,000 SNPs (Reiman et al. 2007). Discovery of this LOAD susceptibility gene, if replicated, provides new opportunities to investigate LOAD pathogenesis, predisposition, treatment, and prevention. Genome-wide studies using even higher density platforms and compound genetic analyses in sufficiently large samples of well-characterized cases and controls promise to play increasingly important roles in the scientific understanding, evaluation, personalized treatment, and prevention of AD.

Genotype-specific responses of AD patients to a particular drug or combination of drugs has been demonstrated although several studies examining the role of ApoE produced conflicting results. A study of the effect of galantamine on cognitive performances in AD patients correlated it with apoE genotyping (Babic et al. 2004). A significant number of responders (71 %) were observed among apoE4 homozygous patients. The subgroup of apoE4 homozygous patients with AD in its mild to moderate stage may be considered as responders to galantamine. The pharmacogenomics of AD may contribute in the future to optimize drug development and therapeutics, increasing efficacy and safety, and reducing side effects in accordance with the concept of personalized medicine.

Apart from ApoE, ~20 genes are associated with AD. One of these, SORL1, was discovered during an international study that analyzed DNA from > 6,000 persons from an isolated population in the Dominican Republic. The study found that this gene can raise the risk of developing AD three times in this population (Rogaeva et al. 2007). Another identified gene, CALHM1, encodes the essential component of a cerebral Ca2+ channel that controls Aβ levels and susceptibility to

late-onset AD, suggesting a potentially new way to treat or even prevent the disease (Dreses-Werringloer et al. 2008). Genome-wide scans have been used to screen the brain's connectivity pattern and the SPON1 variant at rs2618516 on chromosome 11 (Jahanshad et al. 2013). Older persons who carry the connectivity variant rs2618516 have significantly milder clinical dementia scores.

### *Prospects for the Future Management of AD*

The New York Genome Center, in collaboration with Illumina, started a project in 2012 to conduct whole genome sequencing (WGS) of 1,000AD patients over a period of 4 years in order to understand the genetic basis of susceptibility to AD, which will help to assess an individual's lifetime risk of developing the disease, and better define the molecular pathways responsible for neuronal degeneration. This project is a massive undertaking that involves sequencing 30 billion bases per person for 1,000 patient samples and then comparing these sequences to those from normal elderly individuals. Understanding the molecular basis of neuronal degeneration will enable development of effective strategies for early detection and targeted treatment. Functional genomics, proteomics, pharmacogenomics, high-throughput methods, combinatorial chemistry and modern bioinformatics will greatly contribute to accelerate drug development for AD.

## Personalized Management of Parkinson Disease

Parkinson's disease (PD) is characterized by progressive degradation of dopaminergic neurons, which results in both cognitive as well as movement disorders. The drug most commonly prescribed for PD, levodopa is a precursor of dopamine. With use of levodopa, a physician titrates dopamine up to an optimal level for movement and some aspects of cognition. However, the part of the nervous system, which is relatively normal, is overdosed making the drug perform aberrantly. That is why some patients react psychotically to levodopa. Knowing the neural bases of these differential effects will enable clinicians to modify the drug dose, or combine levodopa with other drugs, to produce the best outcome for individual patients and avoid such reactions. There is a trend now towards incorporating genetics into clinical studies of therapy for PD to investigate how a person's genetic make-up influences effect of drugs that work by neurochemical intervention.

### *Genomic Basis of Personalized Approach to Parkinson Disease*

Entacapone, a drug used for the treatment of PD, inhibits catechol-O-methyltransferase (COMT) in a dose-dependent, reversible, and tight-binding manner but does not affect other catechol metabolizing enzymes. It enables the reduction

of levodopa dose. Results of clinical studies, however, indicate that COMT genotype seems to be a minor factor in judging the beneficial effects of entacapone administration. If gene polymorphisms that affect the metabolism of antiparkinsonian drugs can be identified, it might assist physicians in prescribing the drug dose that will balance short-term control of tremors with long-term drug side effects that eventually render PD untreatable.

Results of the largest case-control genome-wide association study so far indicate a substantial contribution of genetics to susceptibility for both early-onset and late-onset PD, although most of the genetic components of this disease remain to be discovered (Do et al. 2011). Understanding of the genomics of PD has been improved by application of molecular methods. Five genes are now known to cause monogenic forms of PD and these were identified using genetic linkage approaches, which require large pedigrees with affected as well as unaffected individuals. Two of these genes, SNCA and LRRK2, cause dominant forms of PD, while mutations inPARK2, PINK1 and DJ-1 were shown to underlie recessive forms of the disease. Eleven loci were identified as risk factors for the development of common forms of PD (Plagnol et al. 2011). However, a significant proportion of inherited cases of PD still remain unexplained genetically and the cause of the disease remains somewhat elusive. Currently, there is no diagnostic test that can confirm PD. Diagnosis is usually made by clinical observation and confirmed only post-mortem by neuropathological studies.

Exome sequencing has now been applied to PD research and has the potential for use as a screening method to identify pathogenic mutations in some PD patients (Bras and Singleton 2011). A major challenge of exome sequencing is the amount of data generated, and the rapid evolution of methods to evaluate these data.

Members of the International Parkinson's Disease Genomics Consortium have used whole exome sequencing (WES) as part of their ongoing search for new genetic contributors to neurodegenerative disease. In 2013, scientists at VU University Medical Center in the Netherlands outlined their efforts and findings. By sequencing and comparing the exomes of individuals with familial or sporadic PD and unaffected controls, they identified common variants, rare variants, and combinations of the two that contribute to risk of PD. They found that over-representation of genes from three inter-connected pathways contribute to mitophagy, autophagy, and endocytosis-related processes. The results indicate that dysfunctions affecting those pathways may contribute to development of PD rather than being a consequence of it. The researchers plan to do array-based profiling on more PD patients using the NeuroX exome chip to verify genetic results from the current exome sequencing study. Consortium members are also in the process of resequencing apparent PD loci detected through past studies of the condition. The team's analysis of common variant contributors to PD pointed to SNPs at >30 loci, while gene-based association tests highlighted 169 genes with potential PD contributions. The group continues to look for new genetic contributors to PD, while attempting to verify candidate associations from the exome sequencing study using lab models such as *Caenorhabditis elegans* and Drosophila as well as cell cultures generated from human neuronal cells.

## *Role of Pharmacogenetics in Personalizing Therapy of PD*

Cytochrome P450 CYP2D6 enzyme, which metabolizes many drugs, is also involved in the metabolism of dopamine. In studies comparing PD patients exhibiting side effects such as "on-off" phenomenon and dyskinesia (both suggesting favorable response to therapy) with a subgroup of patients showing no such response, only the prevalence of CYP2D6 4 allele was found to differ significantly between the PD patients and control group.

Response of individual patients to levodopa and adverse reactions vary considerably. An understanding of the basis of these differential effects may enable modification of the drug dose, or combination of levodopa with other drugs, to produce the best outcome for individual patients and to avoid such reactions. There is a trend now toward incorporating genetics into clinical studies of therapy for PD to investigate how a person's genetic make-up influences the effect of drugs that work by neurochemical intervention. Approximately 50 % of PD patients treated with L-dopa develop L-dopa-induced dyskinesias in the long term, and the use of pharmacogenetics should be explored in an effort to reduce this complication.

## *Discovery of Subgroup-Selective Drug Targets in PD*

Global gene-expression profiles define the four major classes of dopaminergic (DA) and noradrenergic neurons in the brain. Molecular profiles provide a basis for understanding the common and population-specific properties of catecholaminergic (CA) neurons and will facilitate the development of selective drugs. One goal of such studies is to identify genes that may influence the selective vulnerability of CA neurons in PD. The substantia nigra (SN) is most susceptible to PD pathology, whereas the adjacent ventral tegmental area (VTA) DA neurons are less vulnerable and hypothalamic DA neurons are spared. The sparing of VTA neurons could be mediated by selective expression of neuroprotective factors, including neurotrophic factors, detoxifying enzymes, lipoprotein lipase, etc. There is selective high expression of γ-synuclein in neurons of the SN and in locus coeruleus noradrenergic neurons that degenerate in PD, which may modify the toxic effects of the widely expressed α-synuclein protein. Likewise, selective expression of the $Zn^{2+}$ transporter by the SN and VTA may play a role in the pathophysiology of PD. Low concentrations of $Zn^{2+}$ can exert a cell-protective effect; however, excess of Zn $2^{2+}$ is neurotoxic and has been shown to promote degeneration of midbrain DA neurons. Thus the molecular signatures of the major classes of CA neurons improve our understanding of the characteristic features and functions of these neurons and facilitate the discovery of subgroup-selective drug targets.

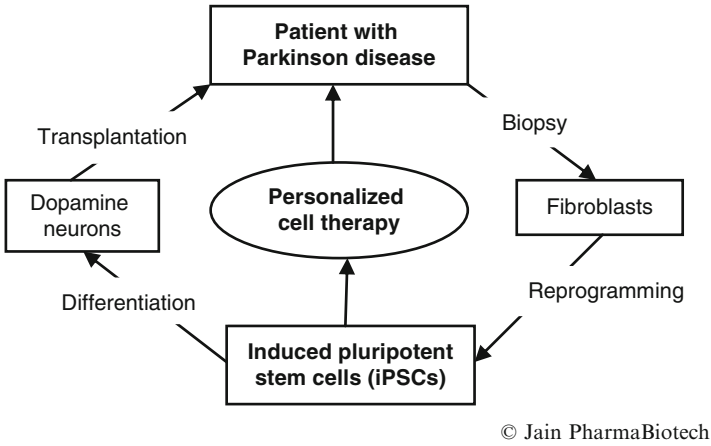


**Fig. 12.3** Scheme of iPSCs for personalized cell therapy of Parkinson disease

### *Personalized Cell Therapy for PD*

Various types of cell therapy have been used for PD but none of these has so far proven entirely satisfactory. Rejection of transplanted cells is one problem. Use of iPSCs for personalized cell therapy of PD is feasible without problem of tissue rejection (Fig. 12.3).

## Personalized Management of Huntington's Disease

### *Genomics of Huntington Disease*

The gene for Huntington disease (HD), which has been cloned, was mapped to the short arm of chromosome 4 using linkage analysis by polymorphic DNA markers. The mutation contains an unstable trinucleotide repeat (cytosine, adenine, and guanine) within a gene in the 4p16.3 chromosome. Because the tip of chromosome 4 contains 50–100 genes, It has not yet been possible to precisely localize the HD gene. Nevertheless, it is known that the disease-causing mutation expands the length of a repeated stretch of amino acid glutamine in the gene's product, the huntingtin protein. Although this development may trigger the onset of HD, other genetic, neurobiological, and environmental factors may also contribute to the progression of the illness and underlying neuronal degeneration. A huntingtin-associated protein has been identified that binds to huntingtin; this binding is enhanced by an expanded polyglutamine repeat, the length of which correlates to the age of disease onset. The huntingtin-associated protein is enriched in the brain, suggesting a possible role for selective brain pathology in HD development.

### *Genetic Testing for HD*

Predictive testing for HD had been available for some time before the HD gene was cloned. In these procedures, polymorphic markers, flanking the HD gene and located some distance from it, were used to track the disease allele through affected pedigrees. This indirect method yielded probabilistic results. Direct mutation analysis of the HD gene is now possible and gives more accurate results. Measurement of the number of cytosine, adenine, and guanine (CAG) repeats in the HD gene represents an effective, direct test with which to confirm the clinical diagnosis in difficult cases.

Genetic testing for HD is a success story so far and should serve as a model for presymptomatic testing of other adult-onset presymptomatic disorders, but there are some errors. The region around and within the CAG repeat sequence in the HD gene is a hot spot for DNA polymorphisms, which can occur in up to 1 % of subjects tested for HD. These polymorphisms may interfere with amplification by PCR, and so have the potential to produce a diagnostic error. Further refinements in diagnostics are desirable.

### *Personalized Cell Therapy for Huntington Disease*

Human iPSCs derived from Huntington disease (HD) patient fibroblasts can be corrected by the replacement of the expanded CAG repeat with a normal repeat using homologous recombination, and that the correction persists in iPSC differentiation into DARPP-32-positive neurons in vitro and in vivo (An et al. 2012). Further, correction of the HD-iPSCs normalized pathogenic HD signaling pathways (cadherin, TGF-β, BDNF, and caspase activation) and reversed disease phenotypes such as susceptibility to cell death and altered mitochondrial bioenergetics in NSCs. The ability to make patient-specific, genetically corrected iPSCs from HD patients will provide relevant disease models in identical genetic backgrounds and is a critical step for the eventual use of these cells in cell replacement therapy.

## Personalized Management of Epilepsy

Epilepsy is mostly a multifactorial disorder although familial forms occur and some epilepsy genes have been identified. With no known intervention to prevent or cure epilepsy, treatment is primarily symptomatic and requires long-term administration of medications to suppress seizure occurrence. Currently, a trial-and-error approach is employed to choose the most effective antiepileptic drug (AED) for a patient from numerous choices, but ~30 % of all patients are resistant to AED therapy, which can be partially attributed to the presence of polymorphisms of genes encoding enzymes involved in AED metabolism.

**Table 12.4** Biomarkers of epilepsy

| |
|---|
| **Gene mutations in genetic epilepsies** |
| **EEG patterns** |
| **MRI biomarkers** |
| **Protein biomarkers** |
| Protein high-mobility group box 1 (HMGB1) |
| **Biochemical markers in blood** |
| Serum prolactin |
| Fas and bcl-2 |
| **Biomarkers in cerebrospinal fluid** |
| Lactate elevation following seizures |
| Metabolites in inborn errors of metabolism with infantile epilepsy |
| Neuron-specific enolase (biomarker for neuronal injury) |
| Cytkines following seizures |
| S100 protein |



## *Biomarkers of Epilepsy*

With so many different types of seizures and causes of epilepsy, there are no universal biomarkers except EEG measurements. Some biomarkers detect diseases that manifest in seizures. There are no characteristic biomarkers of idiopathic epilepsy except those for monitoring seizures and response to treatment. Development of reliable epilepsy biomarkers would be a major advance in personalized management of epilepsy. A classification of biomarkers of epilepsy is shown in Table 12.4.

Genetics of epilepsy is discussed briefly in the following section. Biochemical biomarkers of apoptosis, both the proapoptotic Fas and the anti-apoptotic Bcl-2, are proportionately elevated in sera of patients with idiopathic epilepsy, and their levels are related to the seizure severity and frequency. Pyridoxine deficiency is an uncommon but important cause of intractable seizures presenting in infancy and early childhood. It is usually treated by pyridoxine supplementation. Some children with intractable seizures respond to pyridoxal phosphate rather than pyridoxine, including a rare form of neonatal epileptic encephalopathy shown to be due to mutations in the PNPO gene for pyridox(am)ine 5'-phosphate oxidase. Although the biochemical explanation for this finding is not clear, elevated pipecolic acid levels may serve as a diagnostic biomarker for patients with pyridoxine-dependent seizures. Levels of both pipecolic acid and certain metabolites shown to be elevated in patients with PNPO mutations should be measured, and therapeutic trials of pyridoxal phosphate as well as pyridoxine should be considered early in the course of the management of infants and young children with intractable seizures.

Results of a clinical study show that serum HSP70 levels have an inverse correlation with hippocampal volume after controlling for the effect of age in patients with temporal lobe epilepsy (TLE), and HSP70 is a biomarker for prediction of higher frequencies of seizures in these patients (Chang et al. 2012). HSP70 is considered to

be a stress biomarker in TLE in that it correlates inversely with memory scores and hippocampal volume. In addition, the symmetric extratemporal atrophic patterns may be related to damage of neuronal networks and epileptogenesis in TLE.

High-magnetic-field MRI and long-term video EEG in a rat model of febrile status epilepticus (FSE) has revealed that reduced amygdala T2 relaxation times can predict TLE (Choy et al. 2014). Reduced T2 values likely represent paramagnetic susceptibility effects derived from increased unsaturated venous hemoglobin, suggesting augmented oxygen utilization after FSE termination. Use of deoxyhemoglobin-sensitive MRI sequences enabled visualization of the predictive changes on lower-field, clinically relevant scanners. This novel MRI signature represents a predictive biomarker for early identification of FSE individuals who are likely to develop TLE and are candidates for preventive therapy.

Quantitative measurements by MRI of overall brain volume (gray matter, white matter, and CSF) in temporal lobe epilepsy are clinically meaningful biomarkers that are associated with increased cognitive morbidity. Focal cortical dysplasia (FCD) is a common cause of pharmacoresistant epilepsy that is amenable to treatment by surgical resection. The identification of structural FCD by MRI can contribute to the detection of the epileptogenic zone and improve the outcome of epilepsy surgery. New magnetic resonance-based techniques, such as MR spectroscopy, fMRI, and fMRI/EEG, are more frequently being used to increase the yield of MRI in detecting abnormalities associated with epilepsy.

Noninvasive imaging of brain inflammation would be helpful in determining its role in epileptogenesis and serve as a biomarker for epilepsy. The current imaging toolbox is limited by the range of neuroinflammatory targets that can be visualized directly. Research in this area will further advance as highly specific ligands and reproducible as well as practical imaging approaches become available (Amhaoul et al. 2014).

Molecular and functional interactions between high mobility group box-1 (HMGB1) and the N-methyl-d-aspartate receptor (NMDAR), two proteins playing a key role in neuronal hyperexcitability, have been studied in primary cultures of mouse hippocampal neurons (Balosso et al. 2014). HMGB1 normally resides in the nucleus to regulate transcription, but translocates to the cytoplasm in response to cellular injury and is released into the extracellular milieu where it functions as a pro-inflammatory cytokine biomarker. Disulfide HMGB1 increased phosphorylation of the NR2B subunit of the NMDAR, which is known to increase $Ca^{2+}$ channel permeability and increase NMDA-induced neuronal cell death in vitro and enhance kainate-induced seizures in vivo. This novel molecular neuronal pathway activated by HMGB1 could be targeted in vivo to prevent neurodegeneration and seizures mediated by excessive NMDARs stimulation.

## *Genetics/Genomics of Epilepsy*

Considerable information is being generated by advances in genomic technologies. Integration of these techniques with functional biology and bioinformatics will improve our understanding of the genetic contribution to epilepsy, use of genetic

testing for risk assessment and personalized treatment (Kearney 2012). Personalized WES is already available and WGS is likely to be routinely available within next few years.

Breakthroughs are needed in the identification of new molecular targets that will translate to novel intervention approaches. Discovering genetic variants that increase the susceptibility to disease is a promising avenue to identifying such targets. However, early candidate gene-based studies in epilepsy proved ineffective in identifying genetic risk factors for the non-Mendelian, complex epilepsies, which represent >95 % of clinically encountered epilepsy. Furthermore, genome wide association studies (GWAS) of epilepsy patients have been largely negative, with the exception of several putative susceptibility loci discovered in Han Chinese focal epilepsy and European Caucasians. Results of these GWAS suggest that, similar to other common diseases, associations with SNPs appear likely to account for a small fraction of the heritability of epilepsy, thus fueling the effort to also search for alternative genetic contributors, with a recent increased emphasis on rare variants with larger effects. It is possible that both common and rare variants contribute to an increased susceptibility to common epilepsy syndromes.

Examples where genetic factors play a role in epilepsy are Dravet syndrome, febrile seizures, and epileptic encephalopathies. Dravet syndrome is a rare form of infantile epilepsy that is associated with a high incidence of developmental delays and even (sudden unexplained death in epilepsy. Dravet is caused by a genetic defect in the SCN1A gene-affecting sodium channel. Also, there is a rare mutation in the GABARG2 and SCN1B genes (Al-Baradie 2013). The condition can be managed if diagnosed. A combination therapy of stiripentol, valproic acid, clobazam, and topiramate is promising.

Proline-rich transmembrane protein 2 (PRRT2) gene is related to paroxysmal kinesigenic dyskinesia (PKD), infantile convulsions with PKD, and PKD with benign familial infantile epilepsy. A study that screened PRRT2 exons in a cohort of epileptic patients with febrile seizures identified PRRT2 genetic mutations in 18.4 % of patients (He et al. 214). PRRT2 may provide a new drug target for personalized treatment of febrile seizures.

Epileptic encephalopathy (EE) is a heterogeneous group of severe epilepsy disorders characterized by early onset of seizures and cognitive as well as behavioral features associated with ongoing epileptic activity. Two classical forms are infantile spasms and Lennox-Gastaut syndrome. An exome sequencing study has revealed several de novo mutations of which GABRB3 and ALG13 genes show clear statistical evidence of association with epileptic encephalopathy (Epi4K and EPGP Investigators 2013). Other genes with de novo mutations in this cohort include CACNA1A, CHD2, FLNA, GABRA1, GRIN1, GRIN2B, HNRNPU, IQSEC2, MTOR and NEDD4L. It may be difficult to predict with confidence the responsible gene, and the genetic diagnostics in EE in the future will focus on the genome as a whole rather than single genes or even gene panels. Genomic studies of EE have implications for drug development and personalized treatment of EE because many of these mutations appear to converge on specific biologic pathways.

## *Choice of the Right Antiepileptic Drug*

The primary criterion for the selection of antiepileptic drugs (AEDs) is the patient's seizure type. This practice derives largely from drug studies that assess AED effectiveness for specific seizure types rather than the defined causes of seizures. Despite restriction to partial seizures, the response to an investigational AED is quite variable. The reasons for this include: (i) patient-to-patient variation in the metabolism of the AED; (ii) variations in the ability of AED to bind to the target; (iii) variations in the amount of AED target produced by different individuals; and (iv) different pathophysiological events accounting for the same seizure phenotype.

There are several old AEDs and several new drugs have been introduced in the past few years. However, no single AED is clearly superior to others. Causes of variability of effects of AEDs include genetic differences, pathogenesis and severity of epilepsy, age, nutritional status, renal and liver function, concomitant illnesses, and drug interactions. Available evidence suggests that genetic variants from ABCC2 transporter may be associated with an altered response to AEDs. Results of a meta-analysis the literature indirectly suggest possible role of the ABCC2 transporter at the blood brain barrier in altered drug response in patients with epilepsy (Grover and Kukreti 2013). The authors suggest further studies in different ethnic groups to investigate the effects of the ABCC2 haplotypic variants and perform stratified analysis on the basis of different phenotypic covariates.

Physicians try to match a drug to the patient by trial and error. The final choice may take several months and depends on the efficacy and tolerability of adverse effects. However, the problems still remain of adverse side effects and failure to control seizures in more than 30 % of patients, i.e. drug-resistant epilepsy.

## *Pharmacogenomics of Epilepsy*

One of the approaches to optimize AED therapy is pharmacogenomic testing to detect polymorphisms that may affect efficacy, tolerability, and safety of AEDs include variations in the genes encoding drug-metabolizing enzymes such as cytochrome P450, or drug transporters such as MDR1 and MRP2 (Yoshida et al. 2011). SNP studies have shown that polymorphisms of the gene for malic enzyme 2, a mitochondrial enzyme that converts malate to pyruvate and is involved in neuronal synthesis of GABA, predisposes to idiopathic generalized epilepsy. It is also becoming increasingly clear that SNPs play an integral role in variability in both pharmacokinetics and pharmacodynamics of AEDs. Gene expression patterns of children on valproic acid monotherapy differ according to whether they have continuing seizures or remain free from seizures. This information can be used for personalizing AED therapy. The publication of the human genome and increasing sophisticated and powerful genetic tools offer new methods for screening drugs and predicting serious idiosyncratic side effects. Variations of genes that encode drug targets

**Table 12.5** Influence of gene polymorphisms on efficacy of antiepileptic drugs

| Gene/polymorphism | Influence on action of antiepileptic drug | Reference |
|---|---|---|
| ABCB1/T allele of G2677T | Resistance to carbamazepine as a single drug therapy in the treatment of complex partial seizures in Malaysian patients | Subenthiran et al. (2013) |
| ABCC2/G1249A | Decreased risk of resistance to antiepileptic drugs in metaanalysis of studies involving Chinese as well as Caucasian patients | Chen et al. (2014) |
| ABCC2/T allele of rs3740066 | Resistance to effect of carbamazepine in Mexican patients | Escalante-Santiago et al. (2014) |
| ABCC2/ 1249G>A SCN1A/ IVS5-91G>A | Prediction of drug response due to significant association with carbamazepine/oxcarbamazepine resistant epilepsy in Han Chinese patients | Ma et al. (2014) |
| SCN1A/rs3812718 | The frequency of the AA genotype was significantly higher in carbamazepine-resistant Japanese patients | Abe et al. (2008) |
| SCN1A/rs3812718 | In healthy European volunteers GG homozygotes showed increased carbamazepine-induced cortical silent period compared to AA homozygous subjects | Menzler et al. (2014) |



(SCN1A) and drug transport (ABCB1) proteins can influence the efficacy of AEDs as shown in Table 12.5.

The common SCN1A splice-site polymorphism rs3812718 (IVS5N+5 G>A) may contribute to the pathophysiology underlying genetic generalized epilepsies and is associated with electrophysiological properties of the channel and the effect of AEDs that block Na-channels. Studies associating other genes and their variants with seizure control have inherent weaknesses and have not provided unifying conclusions. A better understanding of the genetic influences on epilepsy outcome is the key to developing the much needed new therapeutic strategies for individual patients with epilepsy.

Control of epilepsy with phenytoin can be a difficult and lengthy process because of the wide range of doses required by different patients and the drug's narrow therapeutic index. Similarly, appropriate doses of carbamazepine take time to determine because of the drug's variable effects on patient metabolism and its potential neurologic side effects. People with epilepsy are genetically different from one another, and some of those differences affect their responses to drugs in a predictable manner. A variant of the gene SCN1A, which is found more frequently in patients on the highest doses of phenytoin, has been implicated in many inherited forms of epilepsy. Detection of these gene variants might identify in advance, which patients will need the higher dose and enable a more optimal dose schedule at the start. Otherwise it could take months to get the seizures under control. These new findings provide a direction for a dosing scheme that could be tested in a clinical trial to assess whether pharmacogenetic testing can improve dosing decisions. Transcranial magnetic stimulation is useful for investigating the effects of genetic variants on cortical excitability and pharmacoresponse.

## *Pharmacogenetics of Epilepsy*

More than one-third of the patients experience adverse drug reactions (ADRs) with AED treatment that are related to individual susceptibility, the specific AED used, prescribing physician's skills rather than toxic effects of multiple AEDs (Canevini et al. 2010). Pharmacogenetic factors have been implicated in immune-mediated or hypersensitivity reactions. Most of the ADRs are predictable and mild as well as tolerable and can be alleviated by dose reduction or discontinuation. However, unpredictable severe ADRs that are fatal in some cases have also been reported and cause significant mortality and long-term morbidity (Gaitatzis et al. 2013). ADRs may be a cause for non-compliance or discontinuation of AED therapy.

Polymorphisms of genes for drug metabolizing enzymes such as CYP2C9 and CYP2C19 can influence the efficacy, pharmacokinetics and ADR patterns of AEDs. Polymorphisms of gene encoding CYP2C9 are more likely to be found in patients who required higher dosages of AEDs such as carbamazepine and phenytoin. Variation in genes can affect tolerability, and safety of AEDs. Pharmacogenetic studies, which show that Asian patients with a particular human leucocyte antigen (HLA) allele, HLA-B*15:02, are at a higher risk for Stevens-Johnson syndrome when using carbamazepine, improve our knowledge of how genetic variations affect the treatment of epilepsy (Löscher et al. 2009). Prospective screening for HLA-B*15:02 prior to carbamazepine therapy in patients from South East Asia is widely accepted and could prevent or reduce the incidence of Stevens-Johnson syndrome as well as toxic epidermal necrolysis, which is another hypersensitivity reaction. Pre-prescription screening may be useful for prevention of these serious ADRs, but is not cost-effective.

Available evidence suggests that genetic variants from ABCC2 transporter may be associated with an altered response to AEDs. Results of a meta-analysis the literature indirectly suggest possible role of the ABCC2 transporter at the blood brain barrier in altered drug response in patients with epilepsy (Grover and Kukreti 2013). The authors suggest further studies in different ethnic groups to investigate the effects of the ABCC2 haplotypic variants and perform stratified analysis on the basis of different phenotypic covariates.

No AED treatment guidelines based on pharmacogenetic data are yet available. There is a need for, and an opportunity to, establish standards specific to the conduct of future AED studies to improve the management of epilepsy.

## *Drug Resistance in Epilepsy*

One of the problems with current therapy of epilepsy is development of drug resistance. One third of patients with epilepsy develop resistance to drugs, which is associated with an increased risk of death and debilitating psychosocial consequences. Multiple seizures prior to diagnosis, correlated with epilepsy type as well as intrinsic severity, are risk factors for development of drug resistance.

Neuroinflammation also plays a key role of in the pathophysiology of resistant epilepsy. Furthermore, transporter polymorphisms contributing to the intrinsic severity of epilepsy are providing robust neurobiological evidence on an emerging theory of drug resistance. Because resistance develops to multiple AEDs, the mechanism is likely nonspecific involving drug-efflux transporters such as ATP-binding cassette sub-family B member 1 (ABCB1, also known as MDR1 and P-glycoprotein 170). Lessons learnt from the ABCB1 studies can help guide future association genetics studies for multidrug resistance in epilepsy (Tate and Sisodiya 2007). Use of AEDs that are not ABCB1 substrates, inhibition of ABCB1 or the development of drugs that can evade ABCB1 might improve the efficacy of treatment in some patients with drug-resistant epilepsy. Further studies in this direction might eventually enable the drugs to be tailored to the patient's profile.

Examination of resected hippocampal tissue at surgery from patients with therapy-resistant TLE shows that the mechanism of action of anticonvulsant carbamazepine, i.e. block of voltage-dependent $Na^+$ channels, is completely lost as compared to tissue from patients who still respond to carbamazepine. These data suggest that study of changes in ion channel pharmacology and their contribution to the loss of anticonvulsant drug efficacy in human epilepsy may provide an important impetus for the development of novel anticonvulsants specifically targeted to modified ion channels in the epileptic brain. It is possible to use human tissue for the demonstration of drug resistance in an in vitro preparation, providing a unique tool in the search for novel, more efficient anticonvulsants. Altered expression of subtypes of the $GABA_A$ receptor has also been observed in patients with drug-resistant temporal-lobe epilepsy (Loup et al. 2009). This represents a TLE-specific dysfunction in contrast to stable $GABA_A$-receptor function in the cell membranes isolated from the temporal lobe of TLE patients afflicted with neoplastic, traumatic, or ischemic temporal lesions and can be antagonized by BDNF. These findings may help to develop new treatments for drug-resistant TLE.

Another mechanism underlying drug resistance in epilepsy may be the same as in cancer: a cellular pump called P-glycoprotein, which protects cells from toxic substances by actively exporting the offending compounds. In one case that became resistant to phenytoin, low levels of phenytoin were demonstrated in association with high levels of P-glycoprotein expression, the product of the MDR1 gene. Currently, there are plenty of opportunities to develop personalized antiepileptic medicines because of the wide variations in effectiveness and adverse effect profile of current AEDs.

The "target hypothesis" postulates that alteration in the cellular targets of AEDs leads to a reduction in their sensitivity to treatment. Use-dependent blockade of the fast Na current in dentate granule cells by carbamazepine is lost in hippocampi resected from patients with carbamazepine-resistant temporal-lobe epilepsy, although this finding does not extend to lamotrigine, which has a pharmacologic action similar to that of carbamazepine. Polymorphisms of the SCN2A gene, which encodes the α2 subunit of the neuronal Na channels, were found to be associated with resistance to AEDs in general as well as to those that act on the sodium channels (Kwan et al. 2008). Whether these changes result in reduced sensitivity to antiepileptic drugs that

act on the receptor is unknown. The main weakness of the target hypothesis is its presumption of a working knowledge of the mechanisms of action of AEDs, which remain incompletely understood. The hypothesis cannot account for the observation that patients often have epilepsy that is resistant to multiple drugs with different modes of action, although it cannot be ruled out that alteration in drug targets may play a contributory role.

Once a patient's epilepsy is recognized to be drug resistant, a personalized treatment plan should be formulated to limit any cognitive deterioration or psychosocial dysfunction. Conditions commonly associated with treatment-resistant epilepsy, such as anxiety, depression, and cognitive and memory disturbances, should be recognized and treated.

Surgery is considered as an option in drug-resistant epilepsy and the decision to offer surgical treatment requires an individualized risk-benefit assessment. Several surgical procedures can be performed, depending on the indication (Kwan et al. 2011):

- The vagus-nerve stimulator is implanted as an adjunctive therapy if partial-onset seizures are resistant to AEDs.
- Anterior temporal lobectomy provides long-term relief from seizures in up to 70 % of adults with drug-resistant temporal-lobe epilepsy.
- Resection of structural lesions causing epilepsy, such as glial tumors and vascular malformations, may be curative.
- Palliative procedures, which are intended to disrupt the pathways important for the propagation of epileptiform discharges and thus reduce the frequency and severity of seizures, are considered when resection of the seizure-generating region is not possible. Corpus callosotomy is usually performed in children with clinically significant learning disabilities and severe generalized epilepsy. In hemispherectomy an extensively diseased and epileptogenic cerebral hemisphere is removed or functionally disconnected.

## *An Algorithm for Personalized Management of Epilepsy*

Several stratification approaches to address the therapeutic challenges in epilepsy, take into consideration several investigations including pharmacogenomic and pharmacogenetic studies (Walker et al. 2015). An algorithm used by the author for personalized management of epilepsy is shown in Fig. 12.4.

## *Future Prospects for Management of Epilepsy*

For the future, it is expected that several gene mutations will be identified in epilepsy using techniques such as DNA microarrays for gene expression and sequencing, e.g. those in ion channel genes. Future drugs may be designed specifically according to the electrophysiological dysfunction as personalized medicines for epilepsy.

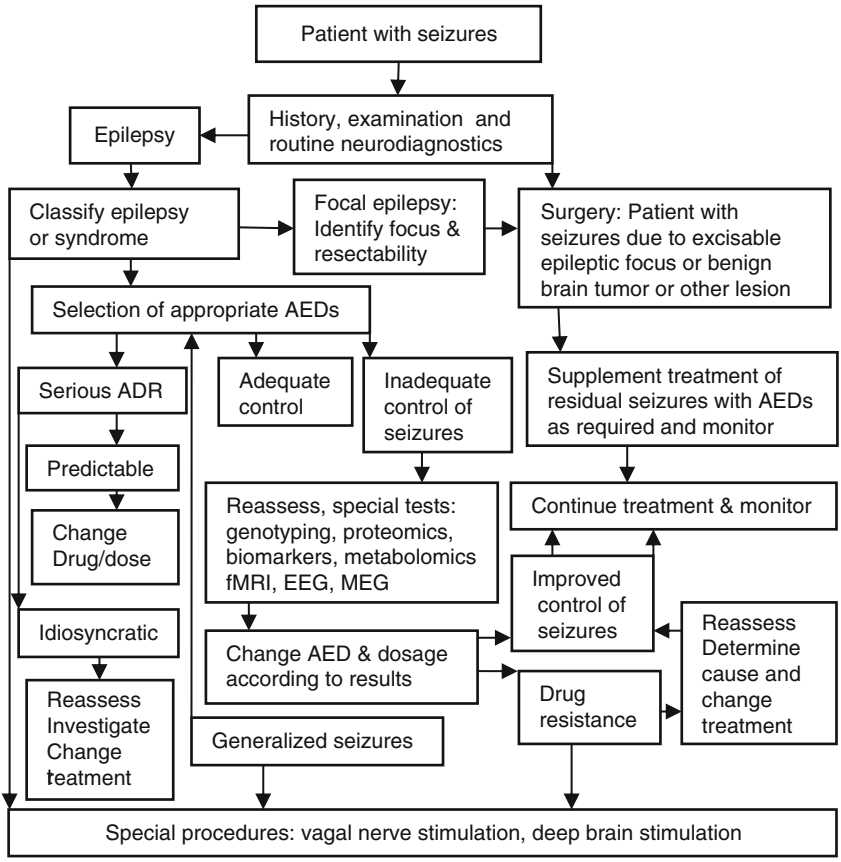




**Fig. 12.4** An algorithm for personalized management of epilepsy

There is ample scope for penetration by new products with a benign side effect profile and/or higher effectiveness. Several new drugs are in development but there is still need for better drugs and strategies to overcome drug resistance.

Study of multidrug transporters is a fruitful area of epilepsy research. The knowledge that multidrug transporters are increased in epileptogenic areas opens potential new avenues for therapeutic intervention. Drugs can be developed to inhibit or bypass overexpressed transporters or implantable devices can be used to deliver high concentrations of drugs directly into the epileptogenic brain parenchyma.

Initial studies have focused on genes whose products play a putatively important role in AED pharmacology, particularly drug transporter proteins, drug metabolizing enzymes and ion channel subunits. However, there is a lack of good correspondence between results from different laboratories, and more recent findings are awaiting attempts at confirmation. Thus, there are currently no AED treatment

guidelines that are based on pharmacogenetic data. Various suggestions that have been made to facilitate development of personalized treatment of epilepsy include the following:

- Standards for the conduct of future AED trials should be established, particularly epilepsy classification, appropriate AED selection, and objective outcome measures.
- Standards for analysis and interpretation of genetic association data must be better codified and applied consistently across studies.
- Extensive clinical research networks should be formed so that large numbers of well characterized patients must be recruited. Neuroimaging techniques, particularly functional MRI (fMRI), as outcome predictors can improve the selection of more suitable treatment options for each patient, e.g. fMRI plays an important role in predicting memory outcome after surgical resections in temporal lobe epilepsy (Yasuda and Cendes 2012).
- Identification of reliable biomarkers to predict response to medical and surgical treatments are much needed in order to provide more adequate counseling about prognosis and treatment options for individual patients. Different neuroimaging techniques may provide combined measurements that may become these biomarkers.

## Personalized Management of Migraine

Migraine is a paroxysmal neurological disorder affecting up to 12 % of males and 24 % of females in the general population. Improvements in prophylactic treatment of migraine patients are desirable because the drugs currently available are not effective in all patients, allow recurrence of the headache in a high percentage of patients and sometimes have severe adverse side effects. Genes involved in neurological, vascular, and hormonal pathways have been implicated in predisposing individuals to migraine. Genetic profiling of predisposition to migraine should facilitate the development of more effective diagnostic and therapeutic applications.

### *Pharmacogenomics of Migraine*

The development of International Hap Map project could provide a powerful tool for identification of the candidate genes in this complex disease and pharmacogenomics research could be the promise for individualized treatments and prevention of adverse drug response (Piane et al. 2007).

The pathophysiology of migraine is not well understood, and although some gene mutations have been associated with special forms of migraine, genetic influences on common migraine at the population level were previously unknown. Mutations in CACNA1A, encoding a neuronal calcium channel subunit, and

ATP1A2, encoding a catalytic subunit of a sodium-potassium-ATPase, have been found in some families with dominantly inherited hemiplegic migraine. These two genes are not associated with more common migraine syndromes and are not the most common hemiplegic migraine genes. However, the work on migraine can also have implications for the increasing number of additional neurological episodic disorders with the common denominator of channelopathy. Genome-wide analysis of a large population in Europe, including migraineurs and non-migraineurs, revealed that two SNPs, rs2651899 and rs10166942, were associated with migraine, but the association was not preferential for migraine with aura or without aura, or with any for specific features of migraine (Chasman et al. 2011).

### *Individualization of Use of Triptans for Migraine*

With a large number of triptans now available, it may be possible to match individual patient needs with the specific characteristics of the individual triptans to optimize therapeutic benefit. Pharmacogenetics provides the possibility of tailoring the therapeutic approach to individual patients, in order to maximize treatment efficacy while minimizing the potential for unwanted side-effects (Buzzi 2008). Pain relief by triptans is significantly modulated by a common genetic variant – G protein beta3 (Schürks et al. 2007). Genetic profiling of predisposition to migraine should facilitate the development of more effective diagnostic and therapeutic applications. Pharmacogenomics will most likely provide a stronger scientific basis for optimizing triptan therapy on the basis of each patient's genetic constitution (Tfelt-Hansen and Brøsen 2008; Tfelt-Hansen 2009).

Pharmacogenomics may help in rationalizing triptan administration according to characterization of an individual's genomic profile. An observational study shows that serotonin transporter gene polymorphism STin2 VNTR confers an increased risk of inconsistent response to triptans in migraine patients (Terrazzino et al. 2010). Although some genetic factors influence drug response, prediction of therapy response with adequate predictive power requires a systematic approach to genetic association studies due to complexity of the field (Gentile et al. 2011).

### *Multitarget Therapeutics for Personalized Treatment of Headache*

Migraine is a special type of headache. For most headaches in practice, genotyping is impractical and unnecessary. Different aspects of pain perception, i.e. sensory and affective components, also explain why there is not just one single target structure for therapeutic approaches to headache. A network of brain areas is involved in pain perception and pain control. This diversification of the pain system explains why a wide range of molecularly different substances can be used in the treatment of

different types of headaches. Most headache medications have more than one component, e.g. combination of acetylsalicylic acid, acetaminophen and caffeine. The major advantage of using such a fixed combination is that the active ingredients act on different but distinct molecular targets and thus are able to act on more signaling cascades involved in pain than most single analgesics without adding more side effects to the therapy. Multitarget therapeutics like combined analgesics broaden the array of therapeutic options, enable the completeness of the therapeutic effect, and enable personalization of treatment to the patient's specific needs. There is substantial clinical evidence that such a multi-component therapy is more effective than mono-component therapies (Straube et al. 2011).

## Personalized Management of Stroke

Stroke accounts for four and a half million deaths each year with an estimated 9 million stroke survivors annually. The overall incidence rate of stroke is 2–2.5 per thousand adults with prevalence of ~5 per thousand and an estimated 5-year risk of stroke recurrence of 15–40 %. Conventional risk factors for stroke include: increasing age, hypertension, diabetes mellitus, smoking, increased body mass index, ischemic heart disease, heart failure, atrial fibrillation and lack of physical activity. Age is the strongest risk factor for both ischemic and hemorrhagic stroke with its incidence doubling for each successive decade after the age of 55 years. However, there is a substantial portion of patients with significant cerebrovascular disease who do not have any of these stroke risk-factors, and it may be helpful to identify complex genetic determinants such as multiple genes that play a role. There is no cure for stroke but principle drugs used currently are antithrombotics and their efficacy and safely can be improved by using pharmacogenetics and pharmacogenomics (Billeci et al. 2009). Personalization of stroke management should start at the stage of clinical trials of various therapies. Stroke treatments may be neuroprotective in the acute stage and neuroregenerative or neurorestorative in the subacute and chronic stages. Various biomarkers and brain imaging can be used to guide clinical trials. Several factors are taken into consideration for personalizing treatment of stroke.

### *Application of Proteomics for Personalizing Stroke Management*

A pharmacoproteomic approach has been proposed for coping with major challenges in translation of stroke research to stratify risk, widen therapeutic windows, and explore novel drug targets. Examples of challenges include thrombolytic treatment for ischemic stroke and treatment for paradoxical embolic stroke related to patent foramen ovale (Ning et al. 2013). In the future, such an approach may help to improve patient selection, ensure more precise clinical phenotyping for clinical trials, and individualize patient treatment.

### *Brain Imaging in Trials of Restorative Therapies for Stroke*

Several restorative therapies for stroke are under investigation including neurotrophic factors, stem cell transplants, small molecules, intensive physiotherapy, robotics, neuroprosthetics, electromagnetic brain stimulation, and mental exercises. Measures of CNS injury and function can be used in many different ways to assist in clinical trials of these therapies. Brain imaging can be a useful approach to select patients and evaluate the efficacy of treatment as well as progress of recovery following stroke (Cramer 2009).

Brain imaging parameters might be used to guide treatment decisions for subjects in a clinical trial. For example, serial imaging measures can be used to individualize details of therapy such as defining dose and duration of treatment, for an individual patient. PET and SPECT have been used to determine response of post-stroke patients to hyperbaric oxygen and help in the decision whether to use hyperbaric oxygen and level of pressure depending on the response to a test treatment (Jain 2009). Imaging measures might also serve as biomarkers of treatment effect. Such data might provide insight into treatment mechanism, which might secondarily guide features of restorative trial design. Changes in laterality or size of motor system activation, revealed by fMRI studies in response to a motor-based therapy, might guide entry criteria to exclude patients with massive motor system injury, and refine restorative stroke trial design.

### *Decisions for Evacuation of Intracerebral Hemorrhage*

Intracerebral hemorrhage (ICH) usually has poor prognosis, which is not altered by surgical evacuation of the hematoma in most cases. Vasculopathic changes associated with the APOE ε2 allele might have a role in the severity and clinical course of lobar ICH. Screening of patients who have ICH to identify the ε2 variant might allow identification of those at increased risk of mortality and poor functional outcomes (Biffi et al. 2011).

### *Decisions for Revascularization Procedures in Chronic Post-stroke Stage*

Patients who have not recovered from stroke in a year despite various treatments and have fixed neurological deficits are usually referred to as being in a chronic post-stroke stage. Because penumbra zone surrounding the cerebral infarct may contain dormant neurons even after a year following the stroke onset, various treatments have been attempted to activate these neurons with the aim of improving cerebral function. Hyperbaric oxygen (HBO) is one of these treatments. Some of these patients undergo extracranial-intracranial (EC/IC) bypass procedure, usually

**Table 12.6** Role of cell therapy in management of stroke according to stage

| Stage of stroke | Duration | Measure required | Methods |
|---|---|---|---|
| Onset | Minutes | Neuroprotection | Hyperbaric oxygen<br>Pharmaceutical |
| Hyperacute | Onset to 6 h | Recanalization of obstructed arteries and restoration of blood flow | Thrombolysis<br>Embolectomy<br>Stenting |
| Subacute | 2–3 days | Start of repair | Cell therapy |
| Chronic | Weeks to years | Regeneration | Hyperbaric oxygen<br>Rehabilitation<br>Surgical revascularization |



a microsurgical anastomosis between the superficial temporal and the middle artery branches, to reroute blood in cases with inoperable cerebral arterial obstruction. The procedure was popular in the 1970s but the results were variable as some patients showed improvement and others not. A multicenter Cooperative study in 1985 reviewed 1,400 patients and concluded that the operation had no advantage over medical management, and was useless. The poorly designed study included many failures where the procedure should not have been done in the first place. The study ignored a 1977 publication in which response to HBO by recovery of neurological deficit in chronic post-stroke stage in 17 out of 35 patients tested was used as a sign of reversibility of cerebral function and an indication for EC/IC bypass and all of these patients improved (Holbach et al. 1977).

## Personalized Cell Therapy for Management of Stroke

Numerous studies provide evidence that hematopoietic stem cells, either after stimulation of endogenous stem cell pools or after exogenous hematopoietic stem cell application (transplantation), improve functional outcome after ischemic brain lesions (Jain 2015b). Various underlying mechanisms include transdifferentiation into neural lineages, neuroprotection through trophic support, and cell fusion. Functional improvement can occur months after stem cell transplantation when the grafted cells have disappeared without histological evidence of replacement of the infarcted tissue and this has been attributed to paracrine effect of stem cells. Several clinical studies employing autologous adult stem cell-based strategies, considered to be a form of personalized therapy of stroke, hold great promise.

## Management of Stroke According to Stage

For a complex disorder such as stroke with several stages and manifestations, it is difficult to specify a single therapy. As a guide to management of stroke from acute to chronic stages, a scheme suggested by the author of this report is shown in Table 12.6.

The methods selected are the considered to be most effective based on evidence available so far. Cell therapy plays an important role in repair and regeneration. Following recanalization of larger arteries by thrombolytic therapy, cell therapy can target residual areas of ischemia due to small vessel atherosclerosis, reduce cell death and facilitate neuronal plasticity as well as regeneration.

Hyperbaric oxygen (HBO), in addition to initial neuroprotection, also enhances mobilization and action of intrinsic as well as transplanted stem cells (Jain 2009). HBO is also an aid to rehabilitation of stroke patients. Response to HBO as transient neurological improvement can also be used as an indication for revascularization procedures such as extra-intracranial bypass operation.

## Personalized Treatment of Multiple Sclerosis

Multiple sclerosis (MS) is a complex disease in which a substantial part of a person's liability to develop the disease is due to a combination of multiple genetic and non-genetic risk factors. Genetic factors are considered to be responsible for the increased frequency of the disease seen in the relatives of individuals affected with MS.

MS is considered to be an autoimmune disease associated with abnormalities in immune regulation. Although etiology and pathogenesis of MS is still controversial, a consistent feature of the pathology of the disease is entry of T cells into the CNS, which induces an autoimmune inflammatory reaction and initiates demyelination. Immunomodulating agents have markedly improved treatment of MS because they reduce the frequency and severity of relapses. Current therapies for MS include interferon-β (IFN-β), glatiramer acetate, natalizumab and chemotherapy. These therapies decrease the number of relapses and partially prevent disability accumulation. However, their efficacy is only moderate and they have adverse effects and that are costly for health systems. The wide heterogeneity of MS as well as different biological responses to immunomodulatory drugs can be expected to contribute to differential treatment responses. Strategies that dissect the relationship between the treatment response and the biological characteristics in individual patients are valuable not only as a clinical tool, but also in leading to a better understanding of the disease. Examples of such approaches are:

1. In vitro and ex vivo RNA expression profiles of MS patients under treatment with IFN-β have been determined by cDNA microarrays. Non-responders and responders to IFN-β as assessed by longitudinal gadolinium-enhanced MRI scans and clinical disease activity differ in their ex vivo gene expression profiles. These findings will help to better elucidate the mechanism of action of IFN-β in relation to different disease patterns and eventually lead to optimized therapy.
2. Spectratyping has shown that T cells receptors (TCRs) are activated in MS patients and Vbeta5.2 expansion is associated with the development of MS. T cell receptor (TCR)-based immunotherapy is feasible for MS patients if it is individualized according to TCR activation patterns of patients at different stages of the disease.

3. The focus in treatment of multiple sclerosis is on neuroprotection, i.e. therapy that stops or slows the progression of the disease in contrast to symptomatic treatment, which may not have any durable effect. Glatiramer acetate, approved for primary progressive form of multiple sclerosis, is a neuroprotective agent. A statistically significant association has been detected between glatiramer acetate response and a SNP in a T-cell receptor beta variant in patients with MS (Grossman et al. 2007).
4. MRI has become established as a reliable, sensitive and reproducible technique for studying the pathophysiology of MS and provides a means for optimizing treatment in for individual patients.
5. Future approaches to multiple sclerosis should integrate clinical and imaging data with pharmacogenomic and pharmacogenetic databases to develop prognostic profiles of patients, which can be used to select therapy based on genetic biomarkers.

## *Genomics of Multiple Sclerosis*

Genome-wide association studies have identified several risk loci, and variation within the major histocompatibility complex (MHC) exerts the greatest individual effect on risk of developing MS. MHC in chromosome 6p21.3 represents by far the strongest MS susceptibility locus genome-wide and was unambiguously identified in all studied MS populations (Oksenberg 2013). Immunologically relevant genes are overrepresented among those mapping close to the identified loci and implicate T helper cell differentiation in the pathogenesis of MS (International Multiple Sclerosis Genetics et al. 2011).

Research is in progress to fully characterize the genes that predispose to MS and modulate its presentation as well as clinical course, which will pose a major challenge in MS genetics research in the coming years. An important advance is functional characterization of the MS risk variant on chromosome 12p13.31 containing the gene TNFRSF1A, which encodes the tumor necrosis factor (TNF) receptor superfamily member 1A with apoptotic activity. TNFRSF1A shows association with MS risk and provides an insight into the pathophysiology that can lead to novel therapeutic strategies (Lill 2014).

## *Pharmacogenetic Approach to Management of Multiple Sclerosis*

A pharmacogenetic research models based on high-throughput single nucleotide polymorphism (SNP) technology have been used to establish the correlation between drug-responsiveness and genetic polymorphisms of MS patients, which may promote the development of personalized medicine for this disease. Spectratyping has shown that T cells bearing particular types of receptors are activated in MS patients. T-cell receptor-based immunotherapy can be applicable to MS

patients if the T cell receptor activation pattern of each patient is determined at different stages of the disease. The BEST-PGx (Betaferon/Betaseron in Early relapsing-remitting MS Surveillance Trial-Pharmacogenomics) has investigated the value of RNA expression profiling and pharmacogenetics in predicting treatment response to interferon beta in patients with early relapsing MS (Kappos et al. 2005).

Genome-wide expression studies in brain tissue and blood samples of MS patients are expected to reveal biomarkers that would help to determine disease course, outcome, or treatment response in early stages of the disease (Habek et al. 2010). An increasing number of genetic polymorphisms have been correlated with MS but so far their relevance to diagnosis of MS is rather low. A large number of genes (including GSTM, IL1B, PD-1, CCR5, OPN, IL4, HLA-DRB1*1501, CD24, ESR1, CD59, CNTF, CRYAB, IFNγ, MEFV, APOE, TGFB1) have been associated with certain MS phenotypes but these correlations are often controversial. Research on pharmacogenomics of MS is increasing but has not produced a useful biomarker for clinical practice so far (Comabella and Vandenbroeck 2011).

## *Immunopathological Patterns of Demyelination for Assessing Therapy*

Early, active multiple sclerosis lesions show several immunopathological patterns of demyelination, which may explain differences in response to therapy in various patients. Therapeutic plasma exchange (TPE) has been successfully used to treat fulminant demyelinating attacks unresponsive to steroids. A Mayo Clinic study demonstrated that patients with pattern II would be more likely to improve after TPE than those with other patterns since pattern II lesions are distinguished by prominent immunoglobulin deposition and complement activation. This is the first evidence that differences in pathological subtypes of MS may predict response to treatment. Correlation of plasma exchange response to tissue pathology supports the hypothesis that different patterns of tissue damage in MS may require different treatment approaches. However, brain biopsies such as those undergone by the patients studied are not routinely done in MS patients. They are only performed for excluding other diagnoses such as tumor or infection. Therefore, it is necessary to identify specific biomarkers from blood, DNA or MRI, which can distinguish between these four patterns without the need for a brain biopsy.

## *Personalizing Mitoxantrone Therapy of Multiple Sclerosis*

Numerous studies have shown that mitoxantrone (MX), an anticancer agent, is highly efficient in suppressing disease activity in multiple sclerosis. It is administered as escalation therapy when other medication no longer suffices and in

extremely severe courses of the disease. The high therapeutic efficacy of this substance, which originates from oncology, is coupled with potential, in part dose-dependent side effects on the heart, reproductive organs, and the bone marrow; thus the pros and cons of its administration must be weighted. Predictors of therapeutic response may result in individualized risk stratification and MX dosing.

It is known that diverse immune cells respond differently to mitoxantrone leading to the hypothesis that specific drug carriers – proteins that eliminate mitoxantrone from the cells – have different influences on different cells as well as on the effectiveness of the drug in different patients. ATP-binding cassette-transporters ABCB1 and ABCG2 represent multi-drug resistance mechanisms involved in active cellular MX efflux. The role of ABC-gene SNPs for clinical MX response has been investigated in multiple sclerosis patients in Germany, corroborated by experimental in vitro and in vivo data (Cotte et al. 2009). It was shown that the differing genetic blueprints of ABC-transporters are indeed linked to the therapeutic response to mitoxantrone. The probability of the patient group with a genetic disposition to low transporter activity responding positively to mitoxantrone is 3.5 times higher than in the group with genetically caused higher transporter activity. The investigators were able to prove that the genetic blueprint of specific transporter proteins allows one to draw conclusions on the effectiveness and risk of side effects of the potent agent mitoxantrone and they hope to be able to develop personalized treatment plans for each patient.

## *Personalized Cell Therapy of Multiple Sclerosis*

### Autologous Bone Marrow Stem Cell Therapy for Multiple Sclerosis

Bone marrow stem cells have been shown in several experimental studies to have beneficial effects in disease models of MS. Safety and feasibility of intravenous, autologous bone marrow cell therapy, without immunosuppressive preconditioning, was tested in a phase I study in six patients with clinically definite, relapsing-progressive multiple sclerosis (Rice et al. 2010). Assessment of efficacy was a secondary objective and employed clinical disability rating scales, multimodal evoked potential (MMEP) recordings, and MRI scans. Cells were harvested, filtered and infused intravenously in a day-case procedure that was well tolerated by patients and was not associated with any serious adverse events. Over a period of 1 year after the therapy, clinical disability scores showed either no change or improvement, and MMEPs showed neurophysiological improvement. MRI scans did not show any significant changes over a post-therapy period of 3 months. The lack of serious adverse effects and the suggestion of a beneficial effect in this small sample of patients with progressive disease justify conducting a larger phase II/III study to make a fuller assessment of the efficacy of mobilization of autologous bone marrow stem cells in patients with MS.

**Fusokine Method of Personalized Cell Therapy of Multiple Sclerosis**

Fusokine (GIFT15), a cytokine prepared by fusion of granulocyte-macrophage colony–stimulating factor (GM-CSF) with interleukin-15 (IL-15), exerts immune suppression via aberrant signaling through the IL-15 receptor on lymphomyeloid cells. This is reverse of immune stimulating effect of either GM-CSF or IL-15 when given singly. Ex vivo GIFT15 treatment of mouse splenocytes converts B cells, normally involved in immune response, into powerful immune-suppressive cells termed GIFT15 Breg cells (Rafei et al. 2009). Unlike T-cells, naturally-occurring immune-suppressing B cells are almost unknown in nature and the idea of using them to control immunity is novel. In this study, mice with experimental autoimmune encephalomyelitis went into complete remission after intravenous infusion of GIFT15 Breg cells paralleled by suppressed neuroinflammation. There were no significant side-effects in the mice and the treatment was fully effective with a single dose. Autologous GIFT15 Breg cells may serve as a new treatment for autoimmune diseases such as multiple sclerosis. Unlike earlier immune-suppressing therapies that rely on drugs, this approach is a personalized form of cellular therapy, which uses the body's own cells to suppress immunity in a more targeted manner. B-cells can be isolated from a patient's blood sample, purified in the laboratory, treated with GIFT15 in a petri dish, and administered back to the patient. Multiple sclerosis should be treated with this method in its earliest stages. Clinical trials are needed to test the treatment's efficacy and safety in humans.

## *Pharmacogenomics of IFN-β Therapy in Multiple Sclerosis*

Affymetrix 100 K SNP arrays have been used to identify 18 SNPs that may explain why some individuals respond better to IFN-β treatment for MS than others (Byun et al. 2008). The study was done on individuals with relapsing-remitting MS over a period of 2 years. Then large-scale pharmacogenomic comparisons were done between those who responded positively to the treatment and those who did not. The researchers found that 18 of the 35 SNPs were significantly associated with positive interferon beta treatment response. Of these 18 mutations, 7 lie within genes and the remainder are in non-coding regions. Many of the detected differences between responders and nonresponders were genes associated with ion channels and signal transduction pathways. The study also suggests that genetic variants in heparan sulfate proteoglycan genes may be of clinical interest in multiple sclerosis as predictors of the response to therapy. Although additional research needs to be done to further validate the study and understand the functional role of IFN-β, the work has the potential to change the approach to MS treatment from a hit-and-miss to a more systematic personalized management.

The BENEFIT (BEtaseron/Betaferon in Newly Emerging multiple sclerosis for Initial Treatment) study incorporated pharmacogenetic and pharmacogenomic

analyses to determine the genetic elements controlling MS. The data from this study suggest that early initiation of treatment with IFN-β1b prevents the development of confirmed disability, supporting its use after the first manifestation of relapsing-remitting multiple sclerosis (Kappos et al. 2007). Expression levels of IFN response genes in the peripheral blood of MS patients prior to treatment could serve a role as biomarker for the differential clinical response to IFN-β (van Baarsen et al. 2008). Biomarkers will enable responders and nonresponders to drugs to be identified, increase the efficacy and compliance, and improve the pharmaco-economic profile of these drugs. Systems biology can be used to integrate biological and clinical data for developing personalized treatment of MS.

Genome-wide expression profiles of peripheral blood mononuclear cells of multiple sclerosis patients within the first 4 weeks of IFN-β administration identified 121 genes that were significantly up- or downregulated compared with baseline, with stronger changed expression at 1 week after start of therapy (Hecker et al. 2012). Eleven transcription factor-binding sites (TFBS) are overrepresented in the regulatory regions of these genes, including those of IFN regulatory factors and NF-κB. TFBS-integrating least angle regression, a novel integrative algorithm for deriving gene regulatory networks from gene expression data and TFBS information, was then applied to reconstruct the underlying network of molecular interactions. A NF-κB-centered sub-network of genes was highly expressed in patients with IFN-β-related side effects.

Understanding of the factors that underlie therapeutic response is the key to identification of predictive biomarkers. Novel developments in pharmacogenomics research are helping to improve the understanding of the pharmacological effects of IFN therapy, and the identification of biomarkers that allow stratification of MS patients for their response to IFN-β (Martinez-Forero et al. 2008). Ultimately, this information will lead to personalized therapy of MS (Vosslamber et al. 2009). Expression of gene as biomarkers of response to IFN-β therapy in multiple sclerosis is shown in Table 12.7.

## *T Cell-Based Personalized Vaccine for MS*

Tcelna® (Opexa Therapeutics) is a T cell-based personalized autologous immunotherapy. It consists of attenuated, patient-specific myelin reactive T-cells (MRTCs) against peptides of the 3 primary myelin proteins: myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP) that have been implicated in T cell pathogenesis of MS. Prior to use, the MRTCs are expanded, formulated, and attenuated (by irradiation) to render them unable to replicate but viable for therapy. These attenuated T cells are administered in a defined schedule of five subcutaneous injections. Patients are expected be treated with a new vaccine series each year based on their altered disease profile or epitope shift. This vaccine is currently in phase III clinical trials.

**Table 12.7** Gene expression as biomarker of response to IFN-β in multiple sclerosis

| Gene | Protein | Function | Comments |
|---|---|---|---|
| CASP3 | Caspase 3 | Regulation of apoptosis | Expression predicts response to IFN-β |
| CAST | Calpastatin | Cell adhesion | Response to IFN-β correlates with gene polymorphisms |
| COL25 | Collagen type XXV | Extracellular proteoglycans | Response to IFN-β correlates with gene polymorphisms |
| FLIP | FLIP | Regulation of apoptosis | Gene expression predicts response to IFN-β |
| GPC5 | Glypican 5 | Ion channel regulation | Response to IFN-β correlates with gene polymorphisms |
| HAPLN1 | Hyaluronan proteoglycan link protein | Extracellular proteoglycans | Response to IFN-β correlates with gene polymorphisms |
| IAP-1, IAP-2 | IAP, IAP2 | Inhibit caspase activation and apoptosis | IFN-β reduces gene expression in B lymphocytes |
| MMP-9 | Matrix metalloproteinase-9 | Disruption of BBB, immune cell migration into the CNS, and myelin degradation | IFN-β suppresses MMP-9 mRNA |
| MX1 | Myxovirus resistance protein 1 | IFN-β-induced protein with an antiviral effect | MX1 is a biomarker of the response to IFN-β |
| STAT | JAK family of proteins | Regulation of expression of genes that mediate biological effects of INF-β | IFN-β activates JAK-STAT signaling pathway |
| TRAIL | TNF-related apoptosis-inducing ligand | Regulation of apoptosis | TRAIL expression is a marker of IFN-β clinical efficacy |



## Personalized Management of Pain

Interindividual differences in the experience of pain have been appreciated clinically for over a century. Essentials of personalized management of pain are shown in Fig. 12.5.

### *Genetic Factors in Response to Pain*

Gender, ethnicity, temperament and genetic factors also contribute to individual variation in pain sensitivity and responses to analgesics. Pain measurement scales can be used differently across individuals based on the past pain experiences of individuals. The outcomes of clinical trials are based on the mean responses of large numbers of subjects but fail to address inter-individual differences.

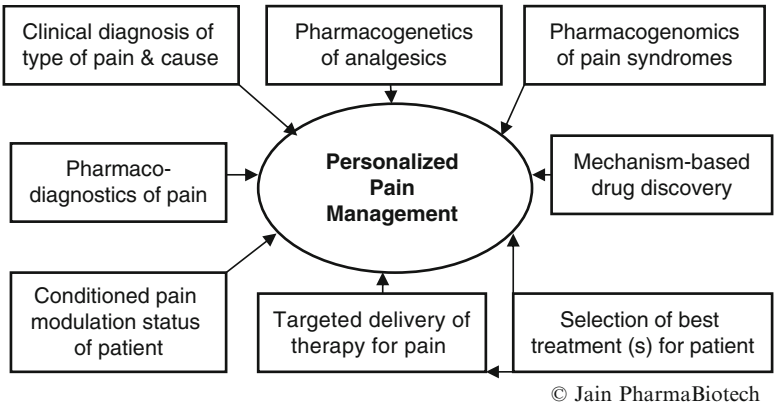


**Fig. 12.5** Essential components of personalized management of pain

The molecular mechanisms that underlie pain vary among individuals over time and among different types of pain to produce wide inter-individual variations in pain perception and response. Pharmacogenetics has the potential to improve patient therapy and care, and it is hoped that it will individualize drug treatment to a greater extent in the near future (Stamer and Stuber 2007).

To address these issues, basic science research is beginning to identify the allelic variants that underlie such antinociceptive variability using a multiplicity of animal models, and powerful genetic approaches are being exploited to accelerate this process. Although the vast majority of these studies have focused on the pharmacogenetics of opioids, owing to their prominent status as analgesics, the number of pharmacotherapies reflecting genetically-based variability is rapidly expanding. In addition, analogous studies have been undertaken in humans, as a small but growing number of clinical trials have begun to evaluate prospectively the existence, often not the origin, of interindividual differences in analgesic drug response. Presentation of the spectrum of individual responses and associated prediction intervals in clinical trials can convey clinically meaningful information regarding the impact of a pain treatment on health-related quality of life. Individual responder analyses are proposed for use in clinical trials to better detect analgesic activity across patient groups and within sub-groups, and to identify molecular-genetic mechanisms that contribute to individual variation.

Studies have shown that people with red hair need 20 % more general anesthesia than blonds and brunettes. Redheads are also more sensitive to thermal pain and are more resistant to the effects of local anesthesia. Variants of the melanocortin-1 receptor (MC1R) gene, which produces melanin, play a role. While blond, brown and black-haired people produce melanin, those with red hair have a mutation of this receptor that produces a different coloring called pheomelanin, which results in freckles, fair skin and red hair. Approximately 5 % of whites are estimated to have these characteristics. Although the relationship between MC1R and pain sensitivity is not proven, MC1R receptors have been found in the brain and some of them are known to influence pain sensitivity.

### *Genetic Mutations with Loss of Pain*

Complete prevention of pain has so far been seen in 6 distinct rare hereditary syndromes: the 'channelopathy-associated insensitivity to pain', caused by 13 currently identified variants in the SCN9A gene coding for the alpha-subunit of the voltage-gated sodium channel, and 5 of the hereditary sensory and autonomic neuropathy (HSAN) I-V syndromes, caused by various mutations in several genes (Oertel and Lötsch 2008). Reduced pain in the average population has been associated with frequent variants in the micro-opioid receptor gene (OPRM1), catechol-O-methyltransferase gene (COMT), guanosine triphosphate cyclohydrolase 1/dopa-responsive dystonia gene (GCH1), transient receptor potential cation channel, subfamily V, member 1 gene (TRPV1) or the melanocortin-1 receptor gene (MC1R). Duplications/amplifications of the cytochrome P450 2D6 (CYP2D6) gene leading to increased enzyme function may cause intense opioid effects of codeine up to toxicity. The COMT V158M variant has been associated with decreased morphine requirements for analgesia. Inactivating MC1R variants have been associated with increased opioid analgesia of the micro-opioid receptor agonist morphine-6-glucuronide and, in women only, of kappa-opioid agonists. Finally, variants in the P-glycoprotein gene (ABCB1) conferring decreased transporter function have been associated with increased respiratory depressive effects of fentanyl. In conclusion, a number of genetic variants that prevent pain by decreasing nociception or increasing analgesia have been identified. Given the complex biological and psychological nature of pain, the interindividual variance in pain and analgesia due to identifiable genetic causes, should be taken into consideration in personalizing pain therapy.

### *Pharmacogenetics/Pharmacogenomics of Pain*

More recently, there has been a growing body of evidence demonstrating differences in analgesic response to various pharmacotherapies, although the source of this variability largely remains to be explained. To this end, basic science research is beginning to identify the allelic variants that underlie such antinociceptive variability using a multiplicity of animal models, and powerful genetic approaches are being exploited to accelerate this process. There is already a growing body of evidence demonstrating differences in analgesic response to various pharmacotherapies, although the source of this variability largely remains to be explained. P450 isoforms involved in the metabolism of some drugs used in the management of pain are shown in Table 12.8.

Although the vast majority of these studies have focused on the pharmacogenetics of opioids, owing to their prominent status as analgesics, the number of pharmacotherapies evincing genetically-based variability is rapidly expanding. In addition, analogous studies have been undertaken in humans, as a small but growing number of clinical trials have begun to evaluate prospectively the existence, if often not the origin, of interindividual differences in analgesic drug response. Presentation of the spectrum of individual responses and associated prediction

**Table 12.8** P450 isoforms in the metabolism of drugs used in the management of pain

| P450 isoforms | Drug category | Examples |
|---|---|---|
| CYP2D6 | Tricyclic antidepressants | Amitriptyline, clomipramine, desipramine |
| | Opioid analgesics | Codeine, tramadol, oxycodone |
| CYP2C9 | NSAIDs | Ibuprofen, diclofenac, naproxen, celecoxib |
| | Antiepileptic drugs | Phenytoin |
| CYP2C19 | Antiepileptic drugs | Phenytoin |



intervals in clinical trials can convey clinically meaningful information regarding the impact of a pain treatment on health-related quality of life. Individual responder analyses in clinical trials can improve detection of analgesic activity across patient groups and within sub-groups, and identify molecular-genetic mechanisms that contribute to individual variation.

Millennium Laboratories' Pharmacogenetic Testing is saliva-based testing to detect genetic variations in enzymes associated with the metabolism of medications commonly prescribed to patients suffering from debilitating chronic pain and pain-related effects. This testing will help clinicians identify patients who may benefit from modifying the drug selection or dosing of certain prescribed analgesics.

Proove Biosciences' Drug Metabolism test offers a proprietary Medication Metabolism Metric to evaluate patients who are slow or fast metabolizers of a drug. Proove Narcotic Risk is a genetic test to identify patients at increased risk for chemical imbalances in the brain that lead to tolerance, dependence, or abuse of prescription pain medications. These tests help select the appropriate analgesic for a patient and reduce the risk of adverse effects and addiction thus facilitating personalized management of pain.

### Pharmacogenetics of Opioids

Although morphine is the analgesic of choice for moderate to severe cancer pain, 10–30 % of patients do not tolerate morphine. Variations in genes involved in mu-opioid receptor signaling influence clinical response to morphine. Codeine analgesia is wholly or mostly due to its metabolism to morphine by the cytochrome P450 enzyme CYP2D6, which shows significant genetic variation in activity. Patients with a mutation in the gene coding for CYP2D6 will show little or no analgesic effect from codeine as it requires a properly functioning CYP2D6 to metabolize it to the active metabolite morphine. Codeine analgesia is less reliable than morphine. Clinically relevant genetic as well as nongenetic factors influencing analgesic responses and side effects of opioids. These are shown schematically in Fig. 12.6.

Catecholamines are involved in the modulation of pain and are partly metabolized by the catechol-O-methyltransferase (COMT) enzyme that degrades catecholamines. Genetic variability in the COMT gene may therefore contribute to differences in pain sensitivity and response to analgesics. It is shown that a polymorphism in the COMT gene, Rs4680 (Val158Met), influences pain sensitivity in human experimental pain

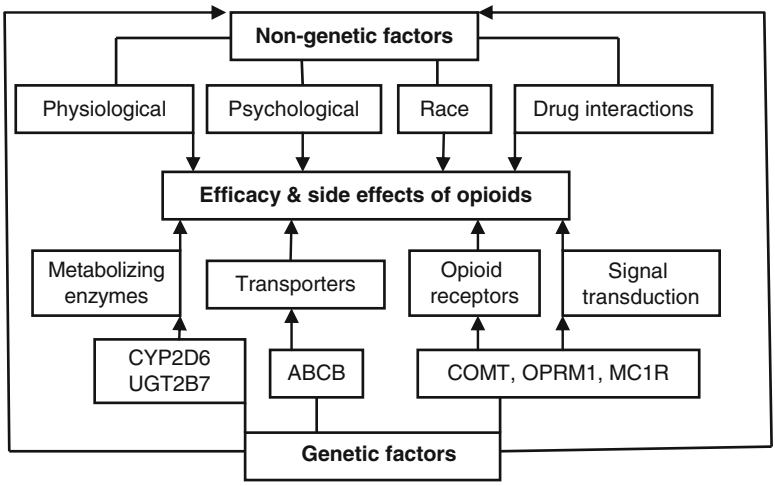


**Fig. 12.6** Genetic & non-genetic factors affecting efficacy and side effects of opioids (Modified from Sadhasivam et al. 2014)

and the efficacy for morphine in cancer pain treatment. Genetic variation in the COMT gene can influence the efficacy of morphine and can explain differences in morphine requirements in individual cancer patients with pain (Rakvåg et al. 2008).

Although available evidence on individual genotype associations with pain, analgesia and opioid adverse outcome are promising, conflicting data in the literature indicates that there is a need for larger and more robust studies with appropriate population stratification and consideration of nongenetic and other genetic risk factors. Relationships between perioperative pain relief in children by opioid drugs and four common SNPs in the COMT gene have been by examined (Sadhasivam et al. 2014). Minor allele carriers of each of these four COMT SNPs, which are predictive of lower COMT activity, were 2.6–3.1 times more likely to require additional analgesic interventions than children homozygotes for the major COMT alleles. These findings may reflect higher CNS catecholamine levels that mediate increased pain sensitivity. This study suggests that application of genotyping can improve surgical pain management in children.

### Pharmacogenetics of NSAIDs

Relief of pain from different NSAIDs varies among patients. It is known that small substitutions in the active site of COX-1, e.g., Ile (isoleucine) for Val (valine), produce the different active site found in COX-2. Therefore, small changes, be they splice variants or mutations, may produce dramatic effects. Mutations such as these might underlie the reason why different patients appear to prefer different NSAIDs. No definite studies have been done on this topic but the phenomenon appears to be widespread as products from approximately one-third of human genes undergo alternative splicing. Different variants from the COX-1 and COX-2 genes could underlie constitutive and inducible prostanoid production. Also, polymorphisms

that alter splice variant expression could predispose patients to differences in disease progression. Genetically defined variations might account for differences of the intensity of inflammatory disease progression.

## *Mechanism-Specific Management of Pain*

The is a need for the development of diagnostic tools that will allow us to identify the mechanisms of pain in an individual patient and pharmacologic tools that act specifically on these mechanisms. This strategy will enable a rational rather than an empirical trial-and-error approach to controlling pain. Treatment with antiinflammatory drugs would be helpful in pain associated with inflammatory conditions but these drugs may not benefit patients whose pain is due mainly due to excitability caused by abnormal sodium channel activity after nerve injury as in painful diabetic peripheral neuropathy.

## *Preoperative Testing to Tailor Postoperative Analgesic Requirements*

Patients vary a great deal in requirement for analgesics after surgery. Determining the best dose for each patient can be difficult because of individual differences in pain tolerance. If patients are undertreated and have severe pain, it can lead to ongoing, chronic pain. On the other hand, over treatment with pain medicine is associated with bothersome side effects.

Research at Wake Forest University Baptist Medical Center (Winston-Salem, NC) shows that having patients complete a series of simple tests before surgery may help predict the intensity of their post-surgical pain and how much pain medication they will need. They conducted a study on women undergoing elective cesarean sections. About 2 weeks before surgery, the women answered questionnaires to measure anxiety, their expectations about pain and the levels of pain they were having during pregnancy. In addition, a small heat element was applied to their arms and backs and the women were asked to rate the intensity and unpleasantness. The heat was not applied long enough to cause skin damage and could be stopped by the patient at any time. After surgery, the women reported on their pain severity levels and researchers measured their requirements for pain medication. The researchers found that six groups of predictive factors accounted for 90 % of the total variances in patients' postsurgical pain severity and medication requirements. The best predictor of the total amount of pain medication required was a validated questionnaire that measured anxiety. The best predictors of overall postsurgical pain were blood pressure readings shortly before surgery and patients' responses to the heat element that was performed before surgery. The model was also useful in identifying patients in the top 20 % of pain severity and amount of pain medication required after surgery. This study shows that it is possible to identify patients at risk for high pain levels after surgery to allow tailored treatments to improve their quality of care.

### *Personalized Analgesics*

Pharmacogenetics has been used in drug development and clinical pharmacology of various diseases but not for pain because the genetic aspects of pain are just beginning to be unraveled. Moreover, the effect of a drug on acute pain and any adverse reaction are apparent immediately, enabling the switching over to another drug. Pharmacogenetics may be applicable in the treatment of some chronic pain syndromes, particularly those with neuropathic pain. Pharmacogenomics, by improving the discovery of analgesic medications and definition of the type of patients for which it would be suitable, will contribute to personalized medicines. Personalized medicines tailored to a patient's needs and selected on a genomic basis are definitely going to be effective and safer, facilitating significant long-term cost savings for the healthcare sector in a managed care environment. This system would enable the selection of an appropriate analgesic for a patient taking into consideration his/her genetic makeup, concomitant disease and comedications. In such a system, two patients presenting with pain due to rheumatoid arthritis may receive different medications.

### *Signature of Pain on Brain Imaging*

Brain imaging studies have been conducted to develop a fMRI-based measure for predicting pain intensity at the level of the individual person (Wager et al. 2013). Machine-learning analyses were to identify a pattern of fMRI activity across brain regions – a neurologic signature – that was associated with heat-induced pain. Pattern included the thalamus, the posterior and anterior insulae, the secondary somatosensory cortex, the anterior cingulate cortex, the periaqueductal gray matter, and other regions. Further studies tested the sensitivity as well as specificity of the signature to pain versus warmth in a new sample and assessed specificity relative to social pain, which activates many of the same brain regions as physical pain. Finally, the responsiveness of the measure to the analgesic agent remifentanil was assessed. The neurologic signature showed sensitivity and specificity of ~94 % in discriminating painful heat from nonpainful warmth, pain anticipation, and pain recall. The strength of the signature response was substantially reduced when remifentanil was administered. The study concluded that it is possible to use fMRI to assess pain elicited by noxious heat in healthy persons. Future studies are needed to assess whether the signature predicts clinical pain and to use it as a guide to development of personalized analgesics.

### *Concluding Remarks on Personalized Management of Pain*

Pain is a complex problem in management. Treatment is guided by the type of pain and response to initial measures. An algorithm is shown in Fig. 12.7 as a simple guide to basics of sequence of various steps for management of inflammatory and neuropathic pain. Details are shown in a special report on pain therapeutics and vary according to type of pain (Jain 2015d). Mechanism-based personalized management of neuropathic pain is shown in Table 12.9.

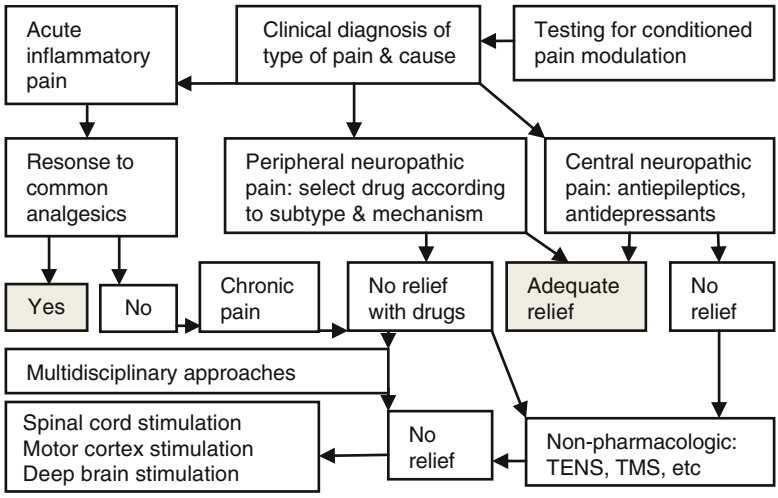


**Fig. 12.7** An algorithm for personalized management of pain

**Table 12.9** Personalized management of neuropathic pain based on mechanism

| Mechanism | Diagnostic features | Molecular targets | Drugs |
|---|---|---|---|
| Altered expression of sodium channels | Spontaneous pain, paresthesias | Na channels sensitive to tetrodotoxin | Local anesthetics<br>antiepileptics<br>antiarrhythmics<br>Tricyclic antidepressants |
| Specific Na channels | Spontaneous pain | Tetrodotoxin resistant Na channels | Selective blockers[a] |
| Central sensitization | Hyperalgesia | NMDA receptor<br>NK 1 receptor<br>nNOS<br>Protein kinase | NMDA antagonist[a]<br>Glycine site antagonists[a]<br>NK1 receptor antagonist[a]<br>nNOS inhibitors[a]<br>Protein kinase inhibitors[a] |
| Peripheral sensitization | Hyperalgesia to nociceptive stimuli | Vanilloid receptor | Capsaicin |
| | | Cannabinoid receptor CB1 | CB1 agonists[a] |
| | Hyperalgesia to thermal stimuli | Neurokinin 1 receptor | Neurokinin 1 receptor antagonist[a] |
| | Neurogenic inflammation | Nerve growth factor | Nerve growth factor antagonists[a] |
| Sympathetic activity | Spontaneous pain | Adrenergic receptors<br>Nerve growth factor (NGF) | Guanethidine, clonidine<br>NGF antagonists[a] |
| Reduced inhibition | Hyperalgesia | Opioid receptors, GABA transaminase, NK 1, adenosine, purine, kainite, cholecystokinin, acetyl choline (nicotinic) | Morphine, gabapentin |

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[a]Drugs in development

## Personalized Management of Traumatic Brain Injury

### *Molecular Basis of Management of Traumatic Brain Injury*

There is considerable variation in the response of patients to traumatic brain injury (TBI). Expression of some genes such as APOE have been implicated in outcome following TBI, but extensive review of literature has revealed contradictory results that are attributable to the heterogeneity of studies. Further research is needed to assess the relationship between genetic traits and clinical outcome of TBI (Davidson et al. 2014). Biomarkers are useful as diagnostic, prognostic, and monitoring adjuncts. Changes in the expression profile of biomarkers such as microRNAs in peripheral blood mononuclear cells may reflect molecular alterations following brain injury that contribute to the sequelae (Pasinetti et al. 2010).

Mild hypothermia, used for neuroprotection following TBI has a significant effect on the gene expression profiles of the hippocampus (Feng et al. 2010). Differential expression of these genes may be involved in the mechanisms of neuroprotection.

## Personalized Management of Sleep Disorders

Sleep is a complex phenomenon in which specific psychological, electrophysiological, neurochemical, endocrinological, immunological and genetic factors are involved. It is obvious that any set protocol of pharmacotherapy is not a satisfactory approach to managing sleep disorders. Polysomnography is the gold standard of sleep investigation, but brain imaging, neuroendocrine testing, DNA sequencing and other laboratory measures can be useful for obtaining a biomarker profile that enable optimization of the effects of individualized therapies while minimizing adverse effects of drugs used to modulate sleep (Dresler et al. 2014).

### *Personalized Therapy of Insomnia*

The treatment of primary insomnia may be complex and clinically challenging. A comprehensive multidimensional evaluation with a thorough history and physical examination coupled with appropriate testing/imaging will facilitate development of a working diagnosis. Optimal treatment strategies of challenging cases typically involve interdisciplinary team approaches (including a sleep medicine specialist) providing multimodal approaches to treatment, including nonpharmacological and pharmacologic strategies. Treatment plans based on sound medical judgment, clinical insight, and a thorough and global understanding of particular patient's comorbid conditions such as depression may lead to optimal patient-specific/patient-focused/

patient centered personalized care. The finding that higher REM density in depressed patients is associated with better response including sleep to Corticotropin-releasing hormone receptor-1 antagonists indicates the importance of using such biomarkers to find the right sleeping drug for the right patient.

## Personalized Approach to Chronic Fatigue Syndrome

Chronic fatigue syndrome (CFS) is a complex illness that includes alterations in multiple body systems and results from the combined action of many genes and environmental factors. Genomic studies including SNPs have linked CFS to five mutations in three genes coding for the glucocorticoid receptor, for serotonin, and for tryptophan hydroxylase that are related to the body's ability to handle stress (Vernon and Reeves 2006). The findings provide evidence of the biological basis of CFS and could lead to improved diagnostic tools and new therapies. Using an integrated genomic approach, a study suggests the possible role for genes involved in glutamatergic neurotransmission and circadian rhythm in CFS and supports further study of novel candidate genes in independent populations of CFS subjects (Smith et al. 2011). Another study used functional and structural equation modeling approaches to assess the contributions of the polymorphism (rs6311), DNA methylation and clinical variables to HTR2A expression in CFS subjects from a population-based study (Falkenberg et al. 2011). Results suggests that rs6311 can affect both transcription factor binding and promoter methylation, and this along with an individual's stress response can impact the rate of HTR2A transcription in a genotype and methylation-dependent manner.

## Personalized Approach to Ataxias

A pilot study has used heterogeneous ataxias as a model neurogenetic disorder to assess the introduction of NGS into clinical practice (Németh et al. 2013). The authors captured several known human ataxia genes by use of NGS in patients with ataxia who had been extensively investigated and were refractory to diagnosis. Pathogenicity was assessed using a bioinformatics approach and novel variants were validated using functional experiments. The overall detection rate in this study was 18 % and varied from 8.3 % in those with an adult onset progressive disorder to 40 % in those with a childhood or adolescent onset progressive disorder. The majority of cases with detectable mutations had a childhood onset but most are now adults, reflecting the long delay in diagnosis. The delays were primarily related to lack of easily available clinical testing, but other factors included the presence of atypical phenotypes and the use of indirect testing. Sequencing was highly efficient and the consumable cost was ~$620. The pathogenicity interpretation pathway predicted numerous mutations in eight different genes: PRKCG, TTBK2, SETX,

SPTBN2, SACS, MRE11, KCNC3 and DARS2 of which nine were novel including one causing a newly described recessive ataxia syndrome. Genetic testing using targeted capture followed by NGS was efficient, cost-effective, and enabled a molecular diagnosis in many refractory cases. A specific challenge of NGS data is pathogenicity interpretation, but functional analysis confirmed the pathogenicity of novel variants. The results have broad implications for neurology practice and the approach to diagnostics.

## Future Prospects of Personalized Neurology

Personalized management of neurological disorders integrates several biotechnologies. Genomics plays an important role although there is inadequate information about relation of gene mutations to some disorders as well as to the action of drugs. Genomic biomarkers for diagnosis may also act as targets for developing personalized therapies. Pharmacogenetics and pharmacogenomics of analgesic drugs is also deserves attention while selecting a drug best suited to an individual patient. Currently, the application of genomics in clinical practice is limited but considerable research is ongoing and routine use in diagnosis and treatment of neurological disorders is expected in the second decade of the twenty-first century.

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# Chapter 13
# Personalized Management of Psychiatric Disorders

## Introduction

Most psychiatric disorders, including schizophrenia, major depression, and bipolar disorder, are considered polygenic. The field of psychiatric genetics has developed considerably in recent years as genome-wide studies have revealed interesting variants. Using SNPs or a small set of SNPs is considered to be an excellent tool to discover genes for psychiatric disorders and potentially an excellent tool for psychopharmacogenetics as well. There are, however, a few obstacles for their use: (1) high-throughput, low-cost genotyping assay systems; (2) definitions of good disease phenotype; (3) a good collaboration effort among geneticists, epidemiologists, and physicians; (4) a good candidate gene(s). Selecting good candidate genes is particularly difficult at the current time, because pathophysiology is unknown in most psychiatric disorders. However, if one can identify a good candidate gene(s), association study using SNPs has more statistical power than linkage analysis. It has been demonstrated that when dealing with a gene that contributes 1–5 % additive effect to phenotype, a huge number of subjects (more than 3,000) is required for linkage study but not for association study. The complexity of the regulation of gene transcription and its interactions with environmental factors implies that straightforward translation of individual genetic information into personalized treatment of psychiatric disorders is unlikely, but integration of data from genomics, proteomics, metabolomics, and biomarkers may enable the development of personalized use of antidepressants (Holsboer 2008).

Before 2008 only a handful of gene variants involved in psychiatric illnesses had been identified, but by 2014 ~200 have been found in the human genome, including common and rare variations and CNVs. Many of these variants (>100) appear to play roles in schizophrenia and autism. Considerable more work needs to done as there may be as many as 8000 gene variations or CNVs involved in schizophrenia.

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Although the basic biology of some of the genes involved in psychiatric disorders is now known, it is still not clear how specific mutations in these genes are actually cause illness.

It is anticipated that psychiatric patients will likely be treated in the near future with drugs that target their illnesses based on specific genetic mutations. Variability of the drug response is a major problem in psychiatry. Between 30 % and 50 % of the patients do not respond adequately to initial therapy and it might several months to find this out. Study of the pharmacogenomic and pharmacogenetic basis of these disorders is important. However, it may take a decade before the knowledge gained from study of genes can be translated into effective therapeutics for the psychiatric patients.

## Psychopharmacogenetics/Psychopharmacodynamics

A particularly important group of pharmacodynamic genes relate to neurotransmitter receptors including serotonin and dopamine. The reason that these are important to consider is that a significant number of drugs used in psychiatry have actions that influence these particular brain chemicals.

### *Serotonin Genes*

Serotonin (5-hydroxytryptamine, 5-HT) appears to play a role in the pathophysiology of a range of neuropsychiatric disorders, and serotonergic agents are of central importance in neuropharmacology. Recently, pharmacogenetic research has begun to examine possible genetic influences on therapeutic response to drugs affecting the serotonin system. At the Department of Psychiatry of the University of Chicago (Chicago, Illinois, USA), genes encoding various components of the 5-HT system are being studied as risk factors in depression, schizophrenia, obsessive-compulsive disorder, aggression, alcoholism, and autism. Genes regulating the synthesis (TPH), storage (VMAT2), membrane uptake (HTT), and metabolism (MAOA) of 5-HT, as well as a number of 5-HT receptors (HTR1A, HTR1B, HTR2A, HTR2C, and HTR5A), have been studied. The critical and manifold roles of the serotonin system, the great abundance of targets within the system, the wide range of serotonergic agents-available and in development-and the promising preliminary results suggest that the serotonin system offers a particularly rich area for pharmacogenetic research.

The serotonin transporter is the molecule that controls the level of serotonin and determines the movement of serotonin between cells. It is influenced by genes that are inherited. An individual with a change in the DNA that encodes the serotonin

transporter may have a reduced ability to move serotonin. Therefore, this person may be less likely to respond to antidepressants that target serotonin and more likely to experience side effects from these medications related to excess serotonin levels.

## *Calcium Channel Gene*

$Ca^+$ channel controls the movement of calcium between cells. There are certain genetic changes that increase the flow of Ca into parts of the brain, producing a higher than normal amount of excitement. An analysis of genome-wide SNP data shows that individual and aggregate molecular genetic risk factors are shared between 5 psychiatric disorders that are treated as distinct categories in clinical practice: autism spectrum disorder, attention deficit hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013). $Ca^+$ channel signaling genes for play a role in all the five disorders.

## *Dopamine Receptor Genes*

The dopamine receptor is a molecule that receives signals from dopamine, a brain chemical that is important for movement and perception. All antipsychotic drugs bind to this receptor and work by blocking the activity of dopamine in parts of the brain. Certain individuals have a genetic variation that can lead to reduced binding attraction between antipsychotic medications and this receptor.

### COMT Genotype and Response to Amphetamine

Catechol O-methyltransferase (COMT) is an enzyme, a molecule responsible for breaking down dopamine and norepinephrine in parts of the brain. The activity of this enzyme is controlled in part by genetic factors. In certain individuals, COMT activity is higher than average, which can lead to increased dopamine breakdown and therefore lower levels of dopamine in the frontal lobe. This may have behavioral consequences, such as difficulty with memory and concentration, as well as experiencing symptoms of depression. A functional polymorphism (val158-met) in the COMT gene has been shown to modulate prefrontal dopamine in animals and prefrontal cortical function in humans. COMT genotype has an effect on response to monoaminergic drugs.

Monamines subserve many critical roles in the brain, and monoaminergic drugs such as amphetamine have a long history in the treatment of neuropsychiatric disorders and also as a substance of abuse. The clinical effects of amphetamine are quite variable, from positive effects on mood and cognition in some individuals, to

negative responses in others, perhaps related to individual variations in monaminergic function and monoamine system genes. Amphetamine enhances the efficiency of prefrontal cortex function assayed with functional MRI during a working memory task in subjects with the high enzyme activity val/val genotype, who presumably have relatively less prefrontal synaptic dopamine, at all levels of task difficulty. In contrast, in subjects with the low activity met/met genotype who tend to have superior baseline prefrontal function, the drug has no effect on cortical efficiency at low-to-moderate working memory load and caused deterioration at high working memory load. These observations illustrate an application of functional neuroimaging in pharmacogenomics and extend basic evidence of an inverted-U functional-response curve to increasing dopamine signaling in the prefrontal cortex. Further, individuals with the met/met COMT genotype appear to be at increased risk for an adverse response to amphetamine.

## *Methylenetetrahydrofolate Reductase*

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that ultimately helps to regulate DNA by turning certain genes on or off. In certain individuals, genetic variations affect the body's ability to turn genes on or off. Some studies have shown an association between changes in the MTHFR gene and schizophrenia, major depression, and cognitive dysfunction such as memory and attention difficulty. Studies have found a link between decreased MTHFR and reduced brain white matter in a part of the brain important for coordination, cognition, and mood.

## *GeneSight Tests for Individualized Therapy of Psychiatric Disorders*

Pharmacogenomics-based GeneSight® technology (Assurex Health Inc) enables tests to guide selection of suitable approved drugs for psychiatric disorders such as depression, anxiety, bipolar disorder, panic disorder, post-traumatic stress disorder, premenstrual dysphoric disorder, obsessive compulsive disorder, and schizophrenia. The following tests are available:

GeneSight Psychotropic. This test analyzes genes that may affect a patient's response to antidepressant and antipsychotic medications. The test includes pharmacokinetic genes from the cytochrome P450 family and pharmacodynamic genes related specifically to the serotonin system.

GeneSight MTHFR. This is a genetic test that can help clinicians determine if additional folic acid supplementation is necessary.

GeneSight ADHD. This test analyzes genes that can affect a patient's response to ADHD medications, including stimulant and non-stimulant medications. The test includes pharmacokinetic genes from the cytochrome P450 family and pharmacodynamic genes related to the regulation of neurotransmitters.

## *Personalized Antipsychotic Therapy*

Although considerable advances have taken place in the pharmacotherapy of schizophrenia, 30–40 % of schizophrenic patients do not respond to antipsychotic treatment and ~70 % of them develop side effects. This variability in treatment response may have a genetic origin in two areas:

1. Genetic mutations in metabolic enzymes can render them inactive and result in the toxic accumulation of drugs or drug metabolites.
2. Genetic variation in drug-targeted neurotransmitter receptors can influence their binding and functional capabilities, affecting the efficacy of the treatment.

Combination of genetic information in drug dynamic and kinetic areas can be used to predict treatment response. Pretreatment prediction of clinical outcome will have a beneficial impact on psychiatric treatment. SureGene LLC is developing AssureGene test, a DNA-based diagnostic tests for schizophrenia, to help personalize the treatment for this condition. Personalized antipsychotic treatment will improve recovery and diminish drug-induced side effects. Further investigations on gene expression and gene-environment interactions will improve the accuracy of the predictions.

It is possible to predict the clinical response to an antipsychotic drug such as clozapine. Several liver cytochromes such as CYP1A2 and CYP3A4 are involved in clozapine metabolism and interindividual variations in plasma levels of this drug are known, CYP1A2 knockout mice have been created to investigate the effect of CYP1A2 for metabolism of clozapine. Such mice have a significant decrease in clozapine clearance compared with wild-type mice and prolonged half-life of plasma clozapine suggesting that CYP1A2 is involved in clozapine metabolism in an animal model. Association studies in multiple candidate genes have been carried out to find polymorphisms that predict response to clozapine in schizophrenia patients. Based on clozapine binding profiles, 19 dopamine receptor polymorphisms, serotonin receptor polymorphisms, histamine receptor polymorphisms, and adrenergic receptor polymorphisms have been studied. A combination of receptor polymorphisms predicted antipsychotic medication response, and their research shows great potential for this mechanism. Clozapine has demonstrated superior efficacy, but because of potential serious side effects and necessary weekly blood monitoring, psychiatrists are sometimes hesitant to use it. However, as this study shows, if one is able to predict clozapine's response in advance, more patients will benefit from its use. This research method also will be applied to other antipsychotic medications. In the future, simple psychopharmacogenetic tests will improve antipsychotic medication treatment as well as its application among individuals.

The ability of dopamine receptor polymorphism to predict clinical response to clozapine has been studied using PET (positron emission tomography). Studies with PET using FDG (18F-fluorodeoxyglucose) and dopamine D3 receptor polymorphism in the promoter region for genetic association study have shown significant metabolic decrease in the frontal and temporal lobes, basal ganglia, and thalamus overall. The clinical responses can be correlated with genotypes. The approach of combining pharmacogenetics and imaging techniques offers the potential for understanding clinical response to treatment and may predict side effects.

**Table 13.1** Enzymes that metabolize antipsychotics

| Drug | CYP2D6 | CYP2C19 | CYP3A4 | CYP1A2 |
|---|---|---|---|---|
| Chlorpromazine | + | | | |
| Clozapine | + | | + | + |
| Fluphenazine | | | | + |
| Haloperidol | + | | + | + |
| Olanzapine | | + | + | |
| Perphenazine | + | | | |
| Risperidone | + | | | |
| Sertindol | + | | | + |
| Thiorodazine | + | + | | |
| Zuclopentixol | + | | | |



Many antipsychotics, including perphenazine, zuclopenthixol, thioridazine, haloperidol and risperidone, are metabolized to a significant extent by the polymorphic cytochrome P450 (CYP) 2D6, which shows large interindividual variation in activity (Dahl 2002). Significant relationships between CYP2D6 genotype and steady-state concentrations have been reported for perphenazine, zuclopenthixol, risperidone and haloperidol when used in monotherapy. Other CYPs, especially CYP1A2 and CYP3A4, also contribute to the interindividual variability in the kinetics of antipsychotics and the occurrence of drug interactions. For many antipsychotics, the role of the different CYPs at therapeutic drug concentrations remains to be clarified. Some studies have suggested that poor metabolizers for CYP2D6 would be more prone to oversedation and possibly parkinsonism during treatment with classical antipsychotics, whereas other, mostly retrospective, studies have been negative or inconclusive. For the newer antipsychotics, such data are lacking. Whether phenotyping or genotyping for CYP2D6 or other CYPs can be used to predict an optimal dose range has not been studied so far. Genotyping or phenotyping can today be recommended as a complement to plasma concentration determination when aberrant metabolic capacity (poor or ultrarapid) of CYP2D6 substrates is suspected. Enzymes that metabolize antipsychotics are shown in Table 13.1. Further prospective clinical studies in well-defined patient populations and with adequate evaluation of therapeutic and adverse effects are required to establish the potential of pharmacogenetic testing in clinical psychiatry.

Receptor Selection and Amplification Technology (ACADIA Pharmaceuticals), a massively parallel, drug discovery engine, is being used to examine possible genetic variations in schizophrenic patient populations that may contribute to differential responses to atypical and typical antipsychotic drugs, i.e. clozapine and haloperidol, respectively. Contributing factors to genetic variation in drug response are determined from these and other studies. Drug discovery programs can be redesigned to mitigate the impact of genetic variation in drug response or alternately clinical trials can be designed to treat only those patients exhibiting genetic

variation that correlates with drug efficacy. Safer and more effective medicines should arise when this information is incorporated into the drug discovery process.

ADRs to antipsychotic therapy constitute another area of concern. The CYP2D6 poor metabolizer phenotype appears to be associated with risperidone ADRs and leads to discontinuation of therapy. This finding was revealed by genetic tests that were performed by allele-specific polymerase chain reaction and/or by the AmpliChip CYP450 microarray system for up to 34 separate CYP2D6 alleles (de Leon et al. 2005). Two logistic regression models with dependent variables (moderate-to-marked ADRs while taking risperidone and risperidone discontinuation due to ADRs) were evaluated with respect to the CYP2D6 phenotype.

Two genes are associated with tardive dyskinesia (a movement disorder) as an adverse reaction to antipsychotic treatment in psychiatric patients: one is dopamine D3 receptor, which involves pharmacodynamics of antipsychotics and the other is CYP1A2, which involves pharmacokinetics of antipsychotics. These two polymorphisms have an additive effect for tardive dyskinesia. These SNPs may be useful for predicting potential side effects from medications.

Risperdal's antipsychotic action is probably mainly explained by the blocking of dopamine receptors, particularly D2 receptors. There are polymorphic variations of this gene DRD2, but it is not clear that they have clinical relevance in predicting adverse drug reactions or antipsychotic response. Previous exposure to antipsychotics increases the need for higher resperidol dosing, but the mechanism for this tolerance is not well understood. Other brain receptors, such as other dopamine, serotonin, and adrenergic receptors may explain some of these adverse drug reactions. Some polymorphic variations in these receptors have been described, but they cannot yet be used to personalize resperidol dosing (de Leon et al. 2008).

## *Personalized Antidepressant Therapy*

Major depressive disorder (MDD), a category of mental illness affecting millions of people worldwide, is one of the leading causes of morbidity and has a significant economic cost. Although the mechanisms of action are not well understood, several antidepressants, including serotonin-selective reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs), have been used for the treatment of depression.

After multiple trials, ~85 % of patients respond to antidepressant treatment. However, only 60–65 % respond to any one drug and response to treatment usually takes 4–8 weeks, if the drug works. A failed first treatment is the best predictor of treatment dropout and treatment dropout is the best predictor of suicide.

Although antidepressant response takes weeks, the effects of antidepressants on monoamine systems is very rapid. Therefore, it is possible that the therapeutic effects of all antidepressants are due to common expression of genes after chronic treatment. The first step toward answering this question is finding out which transcripts are increased or decreased by antidepressant treatment. Such research can be done using an animal model. If a particular system is found to be responsible

**Table 13.2** Enzymes that metabolize antidepressants

| Drug | CYP2D6 | CYP2C19 | CYP3A4 | CYP1A2 |
|---|---|---|---|---|
| Amitripyline | + | + | + | + |
| Nortriptyline | + | | | |
| Imipramine | + | + | + | + |
| Desipramine | + | | | |
| Clomipramine | + | + | + | + |
| Citalopram | | + | + | |
| Fluoxetine | + | | | |
| Fluvoxamine | + | | | + |
| Moclobemid | | + | | |
| Paroxetine | + | | | |
| Sertraline | | | + | |
| Venlafaxine | + | | + | |



for the therapeutic effects of antidepressants, a new antidepressant pharmacotherapy could be developed to activate that system more acutely.

Pharmacogenomic approaches could help in predicting some of these outcomes. Enzymes that metabolize antidepressants are shown in Table 13.2.

A 5-$HT_6$ receptor polymorphism (C267T) is associated with treatment response to antidepressant treatment in MDD. A pharmacogenomic approach to individualize antidepressant drug treatment is based on three levels:

1. Identifying and validating the candidate genes involved in drug-response
2. Providing therapeutic guidelines
3. Developing a pharmacogenetic test-system for bedside-genotyping.

### Biomarkers of Response to Antidepressant Treatment

The most promising biomarkers for response to antidepressant therapy include genetic variants and gene expression profiles, proteomic and metabolomic markers, neuroendocrine function tests, electrophysiology and brain imaging. Incorporation of biomarkers in the treatment of MDD could help improve the efficiency of treatment trials and ultimately speed remission (Breitenstein et al. 2014). Biomarkers of response to antidepressant treatment are shown in Table 13.3.

### EEG to Predict Adverse Effects and Evaluate Antidepressant Efficacy

Changes in brain activity prior to treatment with antidepressants can flag patient vulnerability. Quantitative electroencephalography (qEEG) measures revealed that changes in brain function in the prefrontal region during the 1-week placebo lead-in were related to side effects in subjects who received an antidepressant (Hunter et al. 2005). This study is the first to link brain function and medication side effects, and to

**Table 13.3** Biomarkers of response to antidepressant treatment

| Biotechnology area | Biomarkers |
|---|---|
| Brain imaging | Rostral anterior cingulate cortex activity, hippocampal volume |
| Electrophysiology | Quantitative EEG, REM sleep |
| Gene expression | FK506-binding protein 5 |
| Neuroendocrinology | Dexamethasone/corticotropin-releasing hormone test |
| Pharmacodynamics | SLC6A, 4HTR2A |
| Pharmacokinetics | CYP, ABCB1 |
| Proteomics/metabolomics | BDNF, IGF-1, VEGF |



show a relationship between brain function changes during brief placebo treatment and later side effects during treatment with medication. The findings show the promise of new ways for assessing susceptibility to antidepressant side effects. The ability to identify individuals who are at greatest risk of side effects would greatly improve the success rate of antidepressant treatment. For example, physicians might select a medication with a lower side-effect profile, start medication at a lower dose or choose psychotherapy alone when treating patients susceptible to antidepressant side effects.

A qEEG biomarker, the Antidepressant Treatment Response (ATR) index, has been associated with outcomes of treatment with SSRIs in patients with MDD. In an open study of norepinephrine reuptake inhibitor reboxetine, the ATR index predicted response with 70.6 % sensitivity and 87.5 % specificity, and remission with 87.5 % sensitivity and 64.7 % specificity (Caudill et al. 2014). These results suggest that the ATR index may be a useful biomarker of clinical response during NRI treatment of adults with MDD. Future studies are warranted to investigate further the potential utility of the ATR index as a predictor of noradrenergic antidepressant treatment response.

Having a biological marker of likely treatment effectiveness to predict and guide clinicians' decisions would reduce the likelihood of unsuccessful treatments with antidepressants. The PRISE-MD (Personalized Indicators for Predicting Response to SSRI Treatment in Major Depression) study tested whether qEEG measures taken after 1 week of treatment can predict effectiveness of a full treatment regimen with antidepressant medications. The study, conducted by University of California, Los Angeles, in collaboration with the US National Institute of Mental Health, was completed but no results have been published as of end of 2014.

### Individualization of SSRI Treatment

The introduction of the SSRIs (selective serotonin reuptake inhibitors) has significantly transformed the pharmacological treatment of several neuropsychiatric disorders, particularly of individuals affected by depression, panic disorder, obsessive-compulsive disorder and social phobia. Compared with the previous generation of psychotropic drugs, SSRIs offer an improved tolerability to therapy while maintaining a high level of efficacy. Nevertheless, despite these advantages, not all patients benefit from treatment; some do not respond adequately, while others may

react adversely. This necessitates a review of the initial treatment choice, often involving extended periods of illness while a more suitable therapy is sought. Such a scenario could be avoided were it possible to determine the most suitable drug prior to treatment.

The influence of genetic factors on SSRI efficacy now represents a major focus of pharmacogenetics research. Current evidence emerging from the field suggests that gene variants within the serotonin transporter and cytochrome P450 drug-metabolizing enzymes are of particular importance. It also appears likely that further key participating genes remain to be identified. A study in progress at the Pharmacogenetics Research Network at University of California (UCLA, Los Angeles) is investigating the genetic basis of response to fluoxetine and desipramine among Mexican-Americans, in part by identifying novel SNPs that may be relevant to differing response to antidepressants. The most important areas for future research are exploration of known candidate systems and the discovery of new targets for antidepressants, as well as prediction of clinical outcomes. By comprehensively delineating these genetic components, it is envisaged that this will eventually facilitate the development of highly sensitive protocols for individualizing SSRI treatment.

Genes may influence susceptibility to depression and response to drugs. Since every person has two versions of the serotonin transporter genes, one inherited from each parent, the brain may have only long transporters (ll), only short transporters (ss) or a mixture of the two (ls) Even having one copy of the s gene produces susceptibility to depression and reduced response to SSRIs. Chronic use of 3,4-methyl enedioxymethamphetamine (MDMA, or Ecstasy), a serotonin transporter, is associated with higher depression scores due to abnormal emotional processing in individuals with the ss and ls genotype but not those with the ll genotype (Roiser et al. 2005). These findings indicate that SSRIs probably will not be effective for Ecstasy-induced depression.

The Mayo Clinic (Rochester, MN) is offering a new genetic test through Mayo Medical Laboratories to help US physicians identify patients who are likely to have side effects from drugs commonly used to treat depression. Mayo has obtained a nonexclusive license from Pathway Diagnostics Inc to test for a key genetic biomarker, 5HTT-LPR that identifies people who respond differently to antidepressants, including SSRI, which act specifically by binding to the serotonin transporter, and increase the concentration of the neurotransmitter serotonin in the synapse. These medications include fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), citalopram (Celexa) and escitalopram (Lexapro).

The 5HTT-LPR biomarker has potential to improve management of patients with major depression and others who benefit from SSRI treatment. It provides unique information relating to drug response: side effect and compliance. The ll genotype confers compliance to a SSRI whereas the ss genotype indicates an increased compliance with a noradrenergic and specific serotonergic antidepressant (e.g. mirtazapine). The serotonin transporter genotype assists the physician in making a better choice of antidepressant medications for their patients based upon their serotonin transporter genotype used in conjunction with CYP450 genotyping. Depending upon genotypes, some patients should respond well to SSRIs, some may respond to SSRIs but more slowly, and some patients may respond more effectively to non-SSRI antidepressants.

Usually genetic profiles cannot predict a large percentage of variation in response to citalopram. Data available through the Sequenced Treatment Alternatives to Relieve Depression database was used to create three boosted Classification and Regression Trees to identify 16 subgroups of patients, among whom anticipation of positive or negative response to citalopram was significantly different from 0.5 (Alemi et al. 2011). In a 10-fold cross-validation, this ensemble of trees made no predictions in 33 % of cases. In the remaining 67 % of cases, it accurately classified response to citalopram in 78 % of cases. The authors concluded that for the majority of the patients, genetic biomarkers can be used to guide selection of citalopram. The rules identified in this study can help personalize prescription of antidepressants.

International guidelines for rational therapeutic drug monitoring (TDM) are recognized for personalized treatment with antidepressants and antipsychotics. Retrospective analysis of genotyping of patients with depression suggests a good agreement between the poor metabolism (PM) and ultrarapid metabolism (UM) genotypes, the TDM data and clinical outcome (Sjoqvist et al. 2007). TDM combined with genotyping of CYP2D6 is particularly useful in verifying concentration-dependent adverse drug reactions (ADRs) due to PM and diagnosing pharmacokinetic reasons, e.g. UM for drug failure. This is because ADRs may mimic the psychiatric illness itself and therapeutic failure due to UM may be mistaken for poor compliance with the prescription.

### Role of Protein sFRP3 in Predicting Response to Antidepressants

A Wnt signaling inhibitor, secreted frizzled-related protein 3 (sFRP3), has been identified as a molecular target of antidepressant treatments in rodent models, and revealed the significant association of 3 SNPs in FRZB (the sFRP3 human ortholog) with early antidepressant responses in a clinical cohort (Jang et al. 2013). This protein is the target of both antidepressant drugs and electroconvulsive therapy (ECT). Results of the experiments explain how these therapies likely work to relieve depression by stimulating neural stem cells (NSCs) in the brain to grow and mature. In addition, these experiments raise the possibility of predicting individual's response to antidepressant therapy, and adjusting treatment accordingly. The authors compared gene activity in the brains of mice that had and had not been treated with ECT, looking specifically at genes with protein products that are known to regulate NSCs. The comparison turned up differences in the activity of one inhibitor gene for a chemical chain reaction that had been previously implicated in stimulating NSCs. Specifically, the therapy reduced the amount of protein the inhibitor gene, sFRP3, produced, which would in turn have given the growth-stimulating chain reaction freer rein. To learn more about sFRP3's effects, the team next compared normal mice with mice that had been engineered to lack the sFRP3 protein. They found that the modified mice behaved like normal mice on antidepressants. Moreover, giving antidepressants to the modified mice did not further change their behavior. This strongly suggested that antidepressants work by blocking sFRP3, and without sFRP3, the modified mice had nothing to block.

In order to correlate the findings in mice to what happens in the human brain, the researchers next analyzed genetic information from patients with depression and

tracked their response to a course of antidepressant drugs. They found three common variations in the human version of sFRP3 that were linked to a better response to therapy. Search of a database that correlates gene sequences to gene activity in the human brain revealed that all three variations caused less gene activity. sFRP3 is also regulated by other conditions, including exercise. sFRP3's activity is very sensitive to the amount of activity in the brain. This finding could lead to genetic tests that enable physician's to predict a patient's response to antidepressants, and it also provides a target for potential new therapies for the disease.

### Treatment Resistant Depression

The Massachusetts General Hospital (Boston, MA) started a major study in 2012 aimed at guiding treatment of patients suffering from treatment-resistant major depressive disorder. The study is using genetic biomarker data to compare standard treatment with that guided by Genomind's Genecept assay, which combines a proprietary panel of genetic tests with an analytical report to clinicians. The primary objective of the study is to improve depressive symptoms from baseline to 6 months. Other goals are to change clinician behavior and reduce costs. Researchers will focus on pharmacogenetic genotyping of metabolic activity, which can then be used to guide treatment of patients with antidepressants. Also, genome-wide association study analysis will be performed in the future to identify biomarkers that may be predictive of patient response to and tolerance of certain therapeutics.

### Vilazodone with a Test for Personalized Treatment of Depression

Vilazodone (Clinical Data's VIIBRYD®), a dual SSRI and a 5HT1A partial agonist, is in phase III development in parallel with genetic biomarkers to guide its use as an antidepressant. As approximately one-half of depressed patients do not achieve satisfactory results with current first-line treatment options, a product that combines a genetic test with vilazodone will assist physicians in matching patients with a drug that is more likely to be effective for each patient in the first instance. The primary and supportive secondary efficacy endpoints were met in a randomized, double-blind, placebo-controlled trial. In addition, the study separately identified candidate biomarkers for a potential companion pharmacogenetic test for response to vilazodone.

## Personalized Management of ADHD

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric disorders in children and adolescents. Many different medications are available to treat ADHD, yet little data exists to guide treatment choices, which often must be based on trial and error. Stimulant medications, such as methylphenidate are the most commonly used, effective treatment for ADHD.

### *Genotype and Response to Methylphenidate in Children with ADHD*

Methylphenidate acts primarily by inhibiting the dopamine transporter (DAT), a protein responsible for the reuptake of dopamine from the synapse into presynaptic terminals. However, it is often difficult to predict how patients will respond to ADHD medications.

A double-blinded, crossover trial found that children with a variant form of a dopamine transporter gene, 9/9-repeat DAT1 3′-UTR genotype, responded poorly to methylphenidate in contrast to those with 10/10-repeat variant who showed excellent response (Stein et al. 2005). This study shows that testable genetic differences might be used to predict the effectiveness of methylphenidate in children with ADHD. Further research is needed to determine the mechanisms related to poor response in patients with the 9/9-repeat genotype, and to determine if this group responds differentially to alternative treatments. A larger study is evaluating children with ADHD on two other medications to see if their genes predict who will respond to either or both drugs.

### *Pharmaco-EEG for Personalized Treatment of ADHD*

The 'impaired vigilance' subgroup of ADHD with excess frontal theta or alpha activity on EEG responds well to stimulant medication, whereas in depression this subtype might be unresponsive to antidepressant treatments and respond better to stimulant medication (Arns and Olbrich 2014). A slow individual alpha peak frequency is an endophenotype associated with treatment resistance in ADHD. Future studies should incorporate this endophenotype in clinical trials to investigate further the efficacy of new treatments in this substantial subgroup of patients.

## Personalized Approach to Addiction

### *Pharmacogenetics of Drug Addiction*

Pharmacogenetics provides the tools required to identify genetic predictors of probable drug response, drug efficacy, and drug-induced adverse events-identifications that would ideally precede treatment decisions. Drug abuse and addiction genetic data have advanced the field of pharmacogenetics in general. Although major findings have emerged, pharmacotherapy remains hindered by issues such as adverse events, time lag to drug efficacy, and heterogeneity of the disorders being treated. The sequencing of the human genome and high-throughput technologies are enabling pharmacogenetics to have greater influence on treatment approaches. Genes important in drug abuse pharmacogenetics have been identified, which provide a basis for better diagnosis and treatment of drug abuse disorders.

Since 2007, the National Institute of Drug Abuse (NIDA) has sought SNPs for inclusion in a custom microarray platform to study the genetics and pharmacogenetics of drug abuse, addiction, and related mental disorders. NIDA plans to develop the so-called Neuroarray and is looking for community input on custom SNPs that provide in-depth coverage of genes with prior knowledge of association with drug addiction and related disorders. It intends to make the array available competitively through standard NIH mechanisms to help researchers study genetic vulnerability to addiction and related disorders, and to develop genetic patient profiles for targeted pharmacotherapies.

## *Genetic Polymorphism and Management of Alcoholism*

Several gene variants have been identified as risk or protective factors in alcoholism. The genes coding for dopamine receptors, serotonin transporters, and dehydrogenases represent susceptibility loci for addictive behavior. Polymorphisms of the mu-opioid receptor (OPRM1) and dopamine D4 receptor (DRD4) genes are associated with subjective responses to alcohol and urge to drink. A SNP in the OPRM1 gene has been associated in some studies with the efficacy of naltrexone in reducing drinking, but other studies did not find the same effect. The presence of the L versus the S allele on a serotonin transporter gene has been found to influence responses to ondansetron. Alcoholics with the L-allele have greater alcohol craving than those with the S-allele, and polymorphisms in another receptor result in differences in sensitivity to benzodiazepines used to treat early stage alcohol withdrawal systems.

Alcoholism is a complex psychiatric disorder caused by multiple factors, both genetic and environmental. Furthermore, there are probably different subtypes of alcoholism each with a distinct genetic background, which require different therapeutic approaches. However, gene polymorphisms are not only responsible for a predisposition to alcoholism, but also for the way an individual responds to treatment. Because of the genetic heterogeneity between alcoholics there is no one drug that works in all patients, which has made it necessary to provide multiple treatment options that clinicians can use to find which ones work. A personalized treatment that matches specific interventions to the individual, particularly to an individual's genetic profile, is more efficient.

Topiramate has been shown to reduce drinking and heavy drinking in individuals with alcohol dependence whose goal is to stop drinking. A randomized study has evaluated the efficacy and tolerability of topiramate in heavy drinkers whose treatment goal is to reduce drinking to safe levels (Kranzler et al. 2014). In a European American subsample of the study, topiramate's effect on heavy drinking days was significantly greater than that for placebo only in subjects with SNP rs2832407 in gene GRIK1, which encodes the kainate GluK1 receptor subunit. The moderator effect of rs2832407, if validated, would facilitate the identification of heavy drinkers who are likely to respond well to topiramate treatment and provide an important

**Table 13.4** Genetic influences on pharmacotherapy of alcoholism

| Drug | Genetic variant | Effect on outcome | References |
|---|---|---|---|
| Topiramate | *GRIK1* (rs2832407) | Heavy drinking days<br>Adverse events | Kranzler et al. (2014) |
| Naltrexone | *OPRM1* (Asn40Asp), DRD4 VNTR (rs1799971) | Heavy drinking days<br>Abstinence rates<br>Relapse to heavy drinking | Kim et al. (2009) |
| Ondansetron | LL/LS/SS (5-HTTLPR) (rs1042173), *SLC6A4* (5-HTTLPR) | Drinks per drinking day<br>Days abstinent | Johnson et al. (2011) |
| Sertraline | 5-HTTLPR triallelic *SLC6A4* | Heavy drinking days<br>Drinking days | Kranzler et al. (2011) |
| Acamprosate | *GATA4* (rs1327367) | Relapse | Kiefer et al. (2011) |
| Disulfiram | *DBH* (rs161115) | Adverse events | Mutschler et al. (2012) |

Modified from: Batki and Pennington (2014)

personalized treatment option. New treatment strategies focusing on genes contributing to drug and alcohol dependence (such as gene therapy) have been examined in animal models and clinical trials have been conducted with drugs. Table 13.4 shows genetic influences on pharmacotherapy of alcohol.

However, further research is required before these developments will considerably change today's clinical handling of alcoholism on an individual basis. The NIH/ National Institute on Alcohol Abuse and Alcoholism (NIAAAA) is supporting research in this area. Various human and animal studies can help to determine the full range of genetic variation affecting the pharmacodynamic and pharmacokinetic parameters that result in altered drug efficacy and toxicity. Sequencing technologies to identify variations in candidate genes that may play a role in drug responses, use of pharmacogenetic testing to examine genetic variability in side effects from medication, and use of gene expression profiling to determine transcriptomics changes associated with drug response.

## *Personalized Therapy for Smoking Cessation*

The evidence to date is very consistent with respect to the significance of genetic contributions to smoking behavior. Variants in the genes encoding the α5-α3-β4 nicotinic receptor subunits most strongly contribute to differences in the risk for developing nicotine dependence among smokers and a differential response to pharmacologic treatment for smoking cessation (Bierut et al. 2014). As the field of genetics and smoking research progresses, increasing attention is being devoted to gene-environment interactions, with particular attention to the identification of genetic variants that may modify the effects of pharmacological treatment for smoking.

With advances in molecular biology and genomics technology, individualization of smoking cessation therapy according to genotype is within our grasp. Such research has the potential to improve treatment outcome, thereby reducing morbidity and mortality from smoking-related disease.

### Antidepressant Therapy for Smoking Cessation

It is known that variant alleles of the dopamine receptor D2 (DRD2) gene may play a role in determining nicotine addiction. A dopamine receptor gene polymorphism appears to influence the response of cigarette smokers to smoking cessation therapy that includes an antidepressant medicine – venlafaxine. Individuals with at least one copy of the A1 allele of the DRD2 gene have fewer and less-sensitive D2 dopamine receptors than do individuals with two copies of the A2 allele. A clinical trial showed no significant difference between the active and placebo treatments for the smokers with the A1 allele in terms of reduction in negative affect during their attempt to quit but those with the A2 allele receiving venlafaxine have 25 % lower score on testing for negative affect. This demonstrates the value of genotyping in designing a specific smoking cessation therapy for a subgroup of patients.

### Effectiveness of Nicotine Patches in Relation to Genotype

In women the effectiveness of nicotine patches seems to be related to genotype. Women with the variant T allele of the dopamine D2 receptor DRD2 32806 showed considerable benefit from patches, whereas those with the more common CC genotype did not (Yudkin et al. 2004). The increased effectiveness reflected a tendency to a higher quit rate with the active patches and a lower quit rate with placebo patches. No significant relation between genotype and patch effectiveness was seen for men. The overall effectiveness of nicotine replacement therapy could be greater if the therapy were targeted at those most likely to respond.

## Future Prospects of Personalized Psychiatry

Limited number of applications of personalized medicine approach in psychiatry has shown the usefulness of this approach and identified this as an area for further development. Pre-emptive approaches are an important part of personalized medicine and preventive psychiatry requires predictive tools that are currently not adequate. Biomarkers are needed to develop a clinical staging model for psychiatric disorders. The staging model also facilitates integration of data on the biological, social and environmental factors that influence mental illness into existing clinical and diagnostic infrastructure, which will provide a major step forward in the development of a truly pre-emptive psychiatry (McGorry et al. 2014).

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# Chapter 14
# Personalized Management of Cardiovascular Disorders

## Introduction

The constantly growing volume of available data on pathophysiology of cardiovascular disorders will require an organized interpretation of variations in DNA and mRNA as well as proteins, both on the individual and population level. Advances in biotechnologies are being applied to improve the diagnosis and treatment of cardiovascular disorders (Jain 2011). A five-step strategy can be followed when trying to identify genes and gene products involved in differential responses to cardiovascular drugs (Siest et al. 2007):

1. Pharmacokinetic-related genes and phenotypes
2. Pharmacodynamic targets, genes and products
3. Cardiovascular diseases and risks depending on specific or large metabolic cycles
4. Physiological variations of previously identified genes and proteins
5. Environmental influences on them

## *Cardiogenomics*

The term "cardiogenomics" or "cardiovascular genomics" is applied to the description of genes underlying cardiovascular disorders and the use of genomic technologies for developing diagnosis and treatment of these diseases. Technologies used include traditional molecular biology approaches such as real-time PCR and differential display as well as high-throughput technologies such as microarrays and serial analysis of gene expression (SAGE). Molecular genetic technologies can now provide sensitive and efficient genetic testing, not only to identify polymorphic drug metabolism genes, but also to identify disease-associated genes for diagnosis and risk stratification of many hereditary cardiovascular diseases. A combination of proteomics technologies with genomic technologies has enhanced the understanding of molecular basis of cardiovascular disorders.

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It is estimated that ~12,000 genes are expressed in the cardiovascular system assuming that the total number of genes in the human genome is ~19,000. Reported polymorphisms relevant to cardiovascular disease management are shown in Table 14.1. Genotyping for cardiovascular disorders polymorphisms enables personalization in management.

In patients with systolic dysfunction, the ACE D allele is associated with a significantly poorer transplant-free survival. This effect is primarily evident in patients not treated with β-blockers and is not seen in patients receiving therapy implying that β-blocker therapy can negate this effect. These findings suggest a potential pharmacogenetic interaction between the ACE D/I polymorphism and therapy with β-blockers in the determination of heart failure survival. Further information on this point will be available when a pharmacogenetic substudy of the β-blocker Evaluation of the Survival Trial (BEST) is unblinded. BEST is a randomized, placebo-controlled joint study by the US Veterans Administration and National Heart Lung & Blood Institute that looks at polymorphisms in the genes for ACE, angiotensinogen, angiotensin receptor, $\beta_1$ and $\beta_2$ receptors, and endothelin in over 1,000 patients.

In the familial type of heart failure called dilated cardiomyopathy (DCM), additional mutations have been reported in SCN5A, a gene on chromosome 3. SCN5A encodes the Na ion channel in the heart, which helps regulate transport of positively charged Na ions, and therefore the heart's electrical patterns. This finding broadens the indications for genetic screening of SCN5A beyond isolated rhythm disorders. Since these variations hinder Na transport, it is advisable to avoid using Na channel-blocking drugs in heart failure patients with SCN5A mutations, because those drugs may make the problem worse.

Despite the enormous progress in sequencing the human genome and in molecular genetic and bioinformatic techniques during the past decade, the progress in mapping and identifying genes responsible for complex traits such as coronary heart disease and myocardial infarction has been modest and presents a formidable challenge to medical research in the twenty-first century. One example is the study of why hypertension is more frequent and more severe in Afro-Americans. Although many studies have focused on hypertension in black people in an attempt to understand the genetic and environmental factors that regulate blood pressure, this approach has not been productive. Study of the relationship between specific phenotypes and genotypes, both within and across ethnic groups, is more likely to advance our understanding of the regulation of blood pressure than studies focused on race and blood pressure.

Despite the limitation, impact of genomic analysis on cardiovascular research is already visible. New genes of cardiovascular interest have been discovered, while a number of known genes have been found to be changed in unexpected contexts. The patterns in the variation of expression of many genes correlate well with the models currently used to explain the pathogenesis of cardiovascular diseases. Much more work has yet to be done, however, for the full exploitation of the immense informative potential of cardiovascular genomics. Meanwhile, cardiovascular system is receiving its due share of interest in genomics-based drug discovery and development in the commercial sector.

**Table 14.1** Gene polymorphisms relevant to cardiovascular disease management

| Gene polymorphism | Effect | Significance for management |
|---|---|---|
| ACE (angiotensin converting enzyme) | Deletion allele (ACE D) is associated with increased renin-angiotensin activation of the sympathetic nervous system | For determining responders vs non-responders to β-blockers in heart failure |
| Adducin | Linked to hypertension sodium sensitivity | Response to diuretic therapy and sodium restriction |
| Angiotensin Gene (AGT) | Risk of hypertension | Identifying patients who respond to ACE inhibitors |
| Apolipoprotein (Apo) ε4 | Risk of coronary artery disease. Response to statins | Treatment with simvastatin reduces mortality risk by 50 % in Apo ε4 carriers but only 13 % in Apoε4 non-carriers |
| ATP-binding cassette transporter 1 (ABCA1) | Regulates high-density cholesterol. Risk of coronary artery disease | Target of drugs for controlling hypercholesterolemia |
| Cholesterol ester transferase protein (CETP) | Progression of coronary atherosclerosis. Response to statins | Predicts accelerated atherosclerosis and response to pravastatin therapy in B1B1 carriers but not B2B2 carriers |
| Coronary Heart Disease 1 (CHD 1). | Lipid metabolism | Target for drug development |
| Epithelial sodium channel (β subunit) | Linked to hypertension | Amiloride, a direct antagonist of this channel, is more effective in individuals with this polymorphism |
| Factor VII | Risk of myocardial infarction | Indication for low-dose anticoagulation therapy |
| Interleukin-1 (IL-1) | Inflammatory response in blood vessels and heart disease | Favorable response to statin therapy |
| $PL^{A2}$ polymorphism of gene encoding glycoprotein IIIb | Platelet aggregation and premature myocardial infarction | Patients with this polymorphism should be treated with aspirin, clopidrogrel and statins for prevention of coronary artery disease |
| P-selectin | Unstable angina pectoris | Helps in determining prognosis |
| SCN5A (encodes the Na ion channel in the heart) | Cardiac arrhythmias<br>Dilated cardiac myopathy | Suggests avoidance of Na-channel blockers in patients with SCNA mutations |
| Thrombospondin | Premature coronary artery disease | Indication for anti-platelet and antiinflammatory therapy |
| WNK kinases | Linked to hypertension | Associate signaling pathways provide opportunity for developing targeted antihypertensive therapy |

## Role of Diagnostics in Personalized Management of Cardiovascular Disease

### *Cardiovascular Disorders with a Genetic Component*

Several cardiovascular diseases are recognized to have a genetic component; indeed, a family history of heart disease has always attracted the physician's attention. In recent years, molecular genetics has contributed to the development of molecular cardiology, opening up some new pathways to the diagnosis, prevention, and treatment of some cardiovascular diseases. Genetic approaches have succeeded in defining the molecular basis of an increasing array of heart diseases, such as hypertrophic cardiomyopathy and the long-QT syndrome (Brugada Syndrome), a potentially fatal cardiac disorder associated with serious arrhythmias. Some of the genes that cause cardiovascular diseases are shown in Table 14.2.

Long Q-T syndrome is an inherited form of ventricular arrhythmia in which the interval between the Q and the T waves is longer than normal. This disease reflects a defect in the electrical properties of the cardiac muscle, which predisposes the patient to life-threatening ventricular fibrillation after stress. Five genes have been identified where the mutations are associated with this disorder. These genes encode cardiac potassium ion channels and support the hypothesis that the LQT syndrome results from delayed myocellular repolarization. The diagnosis of long QT syndrome and other channelopathies by an electrocardiogram is often difficult and may be missed, which leaves a patient at risk for sudden cardiac death. FAMILION™ (Transgenomic) is the first commercially available, comprehensive genetic test for a heart rhythm disorder. This DNA test for cardiac ion channel mutations may remove uncertainty for the patients, their families, and their physicians with respect to establishing a diagnosis and can guide the physician in determining the best treatment options for those who are genetically predisposed to potentially fatal cardiac arrhythmias caused by long QT syndrome and related cardiac ion channel diseases. The test examines five cardiac ion channel genes for a mutation that is likely to cause long QT syndrome. If a genetic mutation is detected, its type and location can assist the physician in making treatment selections that could include life-style modification, prescription or avoidance of specific classes of drugs or the implantation of a defibrillator. A patient's family members also benefit from the test because it can identify if they inherited the same mutation as the initially symptomatic patient and may be at risk of a potentially fatal arrhythmia. These relatives often have ambiguous findings on an ECG, while the results of the FAMILION test can answer whether or not they carry the familial mutation.

#### Gene Mutations Associated with Risk of Coronary Heart Disease

Plasma triglyceride levels are heritable and are correlated with the risk of coronary heart disease (CHD). Sequencing of the protein-coding regions of the human genome, the exome, has the potential to identify rare mutations that have a large

**Table 14.2** Genes that cause cardiovascular diseases

| Category | Disease | Gene | Function |
|---|---|---|---|
| Congenital malformations | Atrial septal defect | NKX2-5 | Transcription factor |
| | Holt-Oram syndrome (holes between the atria) | TBX5 | Transcription factor |
| Cardiomyopathy | Familial hypertrophic cardiomyopathy | βmyosin | Muscle contraction (forced generation) |
| | | Troponin T | |
| | | Troponin I | |
| | | Cardiac myosin binding protein C | |
| | | α tropomyosin | |
| | Idiopathic dilated cardiomyopathy | Actin | Muscle contraction (force transduction) |
| | | Dystrophin | |
| Cardiac arrhythmias | Long QT syndrome | KLVQT1 | Potassium channel |
| | | HERG | |
| | | minK | |
| | Idiopathic ventricular fibrillation (Brugada syndrome) | SCN5A | Sodium channel |
| | QT-related cardiac arrhythmia with sudden death | NOS1AP | Gene is regulator of neuronal nitric oxide synthase, which modulates cardiac repolarization |
| Myocardial infarction | Early onset | VAMP8 | Platelet degranulation |
| | Early onset | HNRPUL1 | Encodes a ribonuclear protein |
| Heart failure | Congestive heart failure | KIF6 wild-type gene | Kinesin family member 6 |
| Hypertension | Essential hypertension | AGT | Contraction of arterial smooth muscle |
| Blood lipid disorders | Familial hypercholesterolemia | LDL | Regulation of low-density lipoprotein |
| | Familial dyslipoproteinemias | ApoE | Regulation of plasma lipid concentrations |
| Atherosclerosis | Coronary artery disease | E-S128R | Monitors white blood cell adhesion to the arterial wall |
| | Coronary artery inflammatory disease | Interleukin-1 receptor antagonist (IL-1ra) gene | IL-1ra is a potent natural mechanism for controlling IL-1, and inflammation |
| Thrombotic disorders | Venous thrombosis | Factor V (Leiden mutation) | Procoagulant normally by activated protein C |
| | Stroke | | |

effect on phenotype. Protein-coding regions of genes in participants of European or African ancestry in the Exome Sequencing Project were sequenced to determine whether rare mutations in coding sequence, individually or in aggregate within a gene, were associated with plasma triglyceride levels (Crosby et al. 2014). For mutations associated with triglyceride levels, the investigators evaluated their association with the risk of CHD. Rare mutations in the gene encoding apolipoprotein C3 (APOC3) were associated with lower plasma triglyceride levels. Three of the four mutations that drove this result, were loss-of-function mutations: a nonsense mutation (R19X) and two splice-site mutations (IVS2 + 1G → A and IVS3 + 1G → T). The fourth was a missense mutation (A43T). Approximately 1 in 150 persons in the study was a heterozygous carrier of at least one of these four mutations. Triglyceride levels in the carriers were 39 % lower than levels in noncarriers, and circulating levels of APOC3 in carriers were 46 % lower than levels in noncarriers. The risk of CHD among carriers of any rare APOC3 mutation was 40 % lower than the risk among noncarriers.

Another sequencing study from Denmark found that three rare variants of APOC3 – R19X, IVS2 + 1G → A, and A43T – are associated with substantially reduced levels of nonfasting triglycerides and reduced risk of ischemic cardiovascular disease in the general population (Jørgensen et al. 2014). One limitation of this study is that although the risk of ischemic cardiovascular disease is consistently inversely related to plasma levels of HDL cholesterol in observational studies, clinical trials as well as genetic studies involving mendelian randomization have failed to establish a causal link between plasma levels of HDL cholesterol and the risk of ischemic cardiovascular disease. Nevertheless, the findings of this study are of potential clinical importance, because they suggest that APOC3 is a relevant drug target for reducing residual cardiovascular risk. Inhibition of APOC3 by antisense oligonucleotides was shown to reduce plasma levels of APOC3 and triglycerides in animal models and in a phase I human clinical trial (Graham et al. 2013).

### Gene Variant as a Risk Factor for Sudden Cardiac Death

Extremes of the electrocardiographic QT interval, a measure of cardiac repolarization, are associated with increased cardiovascular mortality. A gene called NOS1AP (CAPON), which may predispose some people to abnormal heart rhythms leading to sudden cardiac death, was identified through a genome-wide association study (Arking et al. 2006). Statistically significant findings were validated in two independent samples of 2,646 subjects from Germany and 1,805 subjects from the US Framingham Heart Study. NOS1AP, a regulator of neuronal nitric oxide synthase (nNOS), modulates cardiac repolarization. The gene, not previously discovered by traditional gene-hunting approaches, appears to influence significantly QT interval length as risk factor for sudden cardiac death. QT interval can be measured noninvasively with an EKG, and each person's QT interval, in the absence of a major cardiovascular event, is stable over time, making it a reliable measure. Approximately 60 % of subjects of European ancestry carry at least one minor allele of the NOS1AP genetic variant, which explains up to 1.5 % of QT interval variation.

Instead of focusing on so-called candidate genes with known functions that are highly suspect in heart beat rhythm, the researchers first focused on people who have extremely long or short QT intervals. They used subjects from two population-based studies, about 1,800 American adults of European ancestry from the Framingham Heart Study of Framingham, Massachusetts, and about 6,700 German adults from the KORA-gen study of Augsburg, Germany. They looked at SNPs that track with having a long or short QT interval. Only one particular SNP correlated with QT interval. That SNP was found near the NOS1AP gene, which has been studied for its function in nerve cells and was not previously suspected to play a role in heart function.

Identifying those at high risk for sudden cardiac death before fatalities occur has been challenging, both at the clinical and at the genetic level. In more than one third of all cases, sudden cardiac death is the first hint of heart disease. It is widely believed that many factors, genetic and environmental, contribute to irregular heartbeat and other conditions that may lead to sudden cardiac death. Now that variants of the NOS1AP gene have been correlated with QT interval length, the next project would be to figure out exactly how the DNA sequence variations alter the function of the gene, and how changes in gene function affects heart rhythm. Being able to identify predisposed individuals can save their lives by prescribing beta-blockers and other drugs that regulate heart rhythm, and even by implanting automatic defibrillators in those with the highest risk.

### KIF6 Gene Test as a Guide to Management of Heart Disease

Carriers of the KIF6 (kinesin family member 6) wild-type gene are 50–55 % more likely to develop coronary heart disease (CHD). KIF6 as a biomarker of CHD is the basis of a genetic test, StatinCheck, developed by Celera and offered through Berkeley HeartLab, which is owned by Celera. It is now licensed by clinical laboratory of Aurora Health Care in Milwaukee, Wisconsin.

Physicians can use the KIF6 test to identify the increased risk for CHD and begin treating their patients with statins. A study investigated whether 35 genetic polymorphisms, previously found to be associated with cardiovascular disease, were associated with MI in the CARE (Cholesterol and Recurrent Events) trial and with CHD in the WOSCOPS (West of Scotland Coronary Prevention Study). In both the CARE and the WOSCOPS trials, carriers of the KIF6 719Arg allele had an increased risk of coronary events, and pravastatin treatment substantially reduced that risk (Iakoubova et al. 2008). Carriers of the 719Arg allele of KIF6 have 34 % higher risk of MI and 24 % higher risk of CHD compared with noncarriers among 25,283 women from the Women's Health Study, confirming and extending previous reports (Shiffman et al. 2008).

CE-marked KIF6 genotyping assay for use on Abbott Laboratories' m2000 real-time PCR platform is available in Europe to detect a genetic biomarker that may be used in conjunction with clinical evaluation and patient assessment to identify individuals at risk for coronary heart disease and to treat patients with elevated cholesterol, for whom statin treatment is being considered.

### NGS Sequencing for Management of Cardiovascular Disorders

Current PCR-based strategies are inadequate for genomic investigations involving many candidate genes. NGS has overcome such limitations so that comprehensive testing is now feasible for clinically complex cases. WGS is currently used only for highly selected, clinically complex cases but with rapidly dropping test costs and increasing accuracy, it will eventually replace most, if not all, currently offered genetic tests (Teekakirikul et al. 2013). A NGS procedure associated with DNA sequence capture has been reported that can sequence 202 cardiomyopathy-related genes simultaneously (D'Argenio et al. 2014). The authors developed a complementary data analysis pipeline to select and prioritize genetic variants. The overall procedure can screen a large number of target genes simultaneously, thereby potentially revealing new disease-causing and modifier genes. By using this procedure, hypertrophic cardiomyopathy patients can be analyzed in a shorter time and at a lower cost than with current procedures. The specificity of the NGS-based procedure is at least as good as other techniques routinely used for mutation searching, and the sensitivity is much better. It will facilitate personalized approach to treatment.

### SNP Genotyping in Cardiovascular Disorders

Illumina has developed a custom SNP biochip for the study of vascular diseases through a collaboration with the Institute of Translational Medicine and Therapeutics (ITMAT) at the University of Pennsylvania, the Broad Institute at MIT, and the National Heart, Lung, and Blood Institute (NHLBI)'s Candidate-gene Association Resource (CARe) Consortium. The IBC chip, named for ITMAT, Broad, and CARe, has been used to analyze SNPs in genes that have been selected for cardiovascular-related phenotypes. Illumina iSelect Custom Genotyping BeadChip can be used to study the genetic diversity of pathways for >2,000 genes that are linked to vascular conditions including hypertension, myocardial infarction, heart failure, stroke, insulin resistance, metabolic disorders, dyslipidemia, and inflammation. The iSelect BeadChip enables scientists to train their research on specific SNPs related to pathways or disease. The microarray will enable researchers to quickly genotype thousands of patients across thousands of genes to identify genetic risk factors underlying vascular diseases and other complex genetic traits.

Typing of specific SNPs in the genome of an individual helps in diagnosing or detecting susceptibility to cardiovascular disease. Common SNPs at 18 loci are reproducibly associated with concentrations of LDL cholesterol, HDL cholesterol, and/or triglycerides. Six of these loci are new, and of these two are associated with LDL cholesterol (1p13 near CELSR2, PSRC1 and SORT1 and 19p13 near CILP2 and PBX4), one with HDL cholesterol (1q42 in GALNT2) and five with triglycerides (7q11 near TBL2 and MLXIPL, 8q24 near TRIB1, 1q42 in GALNT2, 19p13 near CILP2 and PBX4 and 1p31 near ANGPTL3). At 1p13, the LDL-associated SNP is also strongly correlated with CELSR2, PSRC1, and SORT1 transcript levels in human liver, and a proxy for this SNP has been shown to affect risk for coronary

artery disease. A genotype score of nine validated SNPs that are associated with modulation in levels of LDL or HDL cholesterol is an independent risk factor for incident cardiovascular disease (Kathiresan et al. 2008). The score does not improve risk discrimination but modestly improves clinical risk reclassification for individual subjects beyond standard clinical factors.

Fifteen genetic markers of twelve loci have been genotyped in three studies of diabetic patients: the prospective Nurses' Health Study, Health Professional Follow-up Study and the cross-sectional Joslin Heart Study (Qi et al. 2011). Only five SNPs – rs4977574, rs12526453, rs646776, rs2259816, and rs11206510 – showed directionally consistent associations with coronary heart disease (CHD) in the three studies. A genetic risk score (GRS) was created by combining the risk alleles of the five significantly associated loci. Prediction of CHD was significantly improved when the GRS was added to a model including clinical predictors in the combined samples.

### Testing in Coronary Heart Disease

In ischemic heart disease, the patient's arteries have narrowed and the heart cannot pump normally because blood flow (and thus oxygen) is often restricted to the heart muscle. In nonischemic forms of the disease, the heart cannot pump normally because the heart muscle has often enlarged for other reasons, such as physical deformity or alcohol abuse. Both conditions can lead to cardiac arrest or more gradual heart failure as the muscle weakens over time. Differentiation between the two types is important for planning the management. The next step is to develop a test that can be used in a clinical setting. Ischemic patients need to be monitored more closely in case they develop drug resistance and require surgery to unblock clogged arteries. Knowing which patients to treat and how closely to monitor them could significantly improve how well physicians manage the disease and, consequently, improve health outcomes.

Lp-PLA2 (lipoprotein-associated phospholipase A2) is an enzyme that is implicated in the vascular inflammatory pathway that leads to plaque formation and atherosclerosis. Previous hypotheses on the cause of coronary heart disease focused around lipid accumulation within the arterial walls. Increasing evidence now suggests that atherosclerosis is largely an inflammatory disease. The MONICA (MONItoring of trends and determinants in CArdiovascular disease) study showed a statistically significant relationship between elevated Lp-PLA2 and the risk of a coronary event (Koenig et al. 2004). Among individuals in the MONICA population, each standard deviation increase in Lp-PLA2 levels resulted in a 37 % increase in the risk of a coronary event. This study also showed that Lp-PLA2 and C-reactive protein, a biomarker of inflammation, may be additive in their ability to predict risk of coronary heart disease.

Routine cholesterol tests account for only about 50 % of the predictability in heart disease risk. A test based on Vertical Auto Profile (VAP, Atherotech Inc) technology for density gradient ultracentrifugation, which directly measures the cholesterol

content of all lipids, components, and subclasses. VAP is an expanded cholesterol profile that provides direct, detailed measurements of cholesterol, or lipid, subclasses which play important roles in the development of cardiovascular disease. The test identifies twice the number of people at risk for heart disease than traditional cholesterol tests developed in the 1970s. Measurements obtained using. VAP test also provide physicians with a foundation from which to develop individualized treatment plans while continuing to track patients' progress in battling heart disease.

## *Biomarkers and Personalized Management of Cardiovascular Disorders*

The cardiovascular therapeutic area is complex and includes a number of overlapping diseases. In the past, low-cost biomarkers, such as BP and cholesterol measurements were used. However, they do not address issues such as plaque stability and size. Many new biomarkers have been discovered in recent years. Many of these are bases for diagnostic tests and there have potential uses in drug discovery and development. There is need for better diagnostic tests including those encompassing metabolic syndrome – a constellation of disorders including cardiovascular diseases, diabetes, and obesity. Other clinical biomarkers for cardiovascular diseases will include intravascular ultrasound, and in vivo tests for plaque composition and stability using imaging. Biomarkers will be important for development of personalized therapies for cardiovascular disorders.

## *Pharmacogenomics of Cardiovascular Disorders*

Application of pharmacogenomics for development of personalized treatment of cardiovascular disorders is illustrated by a few examples, such as myocardial infarction, heart failure and hypertension, which are common conditions. The application of pharmacogenetics to cardiovascular disease management is also discussed. Factors that may be taken into account when selecting drug therapy for a patient with cardiovascular disease include age, race, concomitant diseases, medications, and renal and hepatic function. The renin-angiotensin system (RAS) plays a major role in the development and progression of cardiovascular diseases by promoting vasoconstriction, sodium reabsorption, cardiac remodeling, norepinephrine release, and other potentially detrimental effects. Angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II type 1-receptor (AT1R) blockers are recommended for managing cardiovascular diseases, such as hypertension, myocardial ischemia and heart failure. However, there is substantial variability in individual responses to these agents.

## *Modifying the Genetic Risk for Myocardial Infarction*

Variants in the 5-lipoxygenase–activating protein (FLAP) gene are associated with risk of myocardial infarction (MI). A randomized, prospective, placebo-controlled, crossover trial of DG-031 (DeCode Genetics Inc), an inhibitor of FLAP, was conducted in MI patients who carry at-risk variants in the FLAP gene or in the leukotriene A4 hydrolase gene (Hakonarson et al. 2005). In patients with specific at-risk variants of two genes in the leukotriene pathway, DG-031 led to significant and dose-dependent suppression of biomarkers that are associated with increased risk of MI events. The investigators, however, do not know whether the drug's ability to suppress the biomarkers of inflammation would translate into a decreased risk of heart attack. There are some uncertainties about the rationale for the drug. One is that although some cardiologists theorize that inflammation is indeed a contributory cause of heart attacks, others regard it as just a symptom. If it is a symptom, a drug that reduced inflammation would do nothing to prevent heart attacks. Further research is needed to confirm the link between the gene variant and heart disease. If the drug proves effective, it could be taken as widely as the statin drugs. The average risk for a man older than 40 of having a heart attack at some time in his life is 49 % and although just 33 % of Americans have the at-risk variant, many more might gain a protective effect from the drug.

## *Personalized Management of Chronic Myocardial Ischemia*

Chronic myocardial ischemia is generally due to one or more significant obstructive lesions in the coronary arteries. Myocardial ischemia leads to a dramatic reduction in myocardial contractility and impaired activity of the ion pumps involved in myocardial contraction-relaxation processes. An early event is an increase in intracellular Na+, mainly induced by an increase in the late Na+ current (INa), which increases action potential duration and impairs Ca++ removal from the cell. High $Ca^{++}$ keeps contractile proteins active, increasing energy consumption and diastolic tone, and impairing ventricular relaxation. This process might create a vicious circle, potentially increasing coronary vessel resistance and decreasing coronary blood flow.

There is still some controversy about selection of medical versus surgical therapy for long-term management of patients with stable chronic myocardial ischemia. Patients with coronary artery disease who have prognostically significant lesions or symptoms despite optimum medical therapy require mechanical revascularization with coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI) or both. CABG has been the predominant mode of revascularization for more than half a century and is the preferred strategy for patients with multivessel disease, especially those with diabetes mellitus, left ventricular systolic dysfunction or complex lesions. There have been significant technical and technological advances in PCI over recent years, and this is now the preferred revascularization modality in patients with single-vessel or low-risk multivessel disease. Improvements in both CABG (including total arterial revascularization, off-pump CABG and 'no-touch' graft harvesting) and PCI (including newer-generation stents, adjunctive pharmacotherapy

and intracoronary imaging) mean that there will a need for some guidelines in selection of the procedure best suited for an individual patient. A 'heart team' approach is strongly recommended to select an evidence-based, yet individualized, revascularization strategy for all patients with complex coronary artery disease (Iqbal and Serruys 2014). Finally, regardless of the method of revascularization, an adjunctive medical therapy is important for all patients with coronary artery disease, and this should also be personalized.

### Management of Chronic Angina Pectoris

Chronic stable angina pectoris represents a major burden for public health systems because of its poor prognosis and high treatment costs. Current pharmacological approaches include short- or long-acting nitrates, Ca-channel blockers and β-blockers. However, even their intensive use is not highly effective in preventing angina.

Ranolazine (Gilead Sciences' Ranexa) is approved for the treatment of chronic stable angina and is a potential antiarrhythmic agent as well. It may play a useful role in the personalized management of ischemic heart disease. Ranolazine is a potent inhibitor of the late Na+ current, and improves oxygen consumption, diastolic dysfunction and coronary blood flow. Its mechanism of action is markedly different from other antianginal drugs. By altering the intracellular Na level, ranolazine affects the Na-dependent Ca channels, and indirectly prevents Ca overload that causes cardiac ischemia. Several randomized, double-blind, placebo-controlled trials provided the evidence that supported the approval of a sustained-release formulation of ranolazine for clinical use in chronic ischemic heart disease (Carbone et al. 2013). Compared with other antianginal drugs, ranolazine provided an anti-ischemic effect without hemodynamic changes such as bradycardia or hypotension. This enables safe use of ranolazine in addition to other drug classes, improving control of anginal symptoms and representing a useful option in the presence of several comorbidities such as diabetes. Treatment with ranolazine was shown to be generally well tolerated, although it remains contraindicated in severe renal failure or moderate to severe hepatic impairment, and also has potential drug interactions through CYP450 enzymes. The particular mechanism of action of ranolazine also confers a potential antiarrhythmic effect, particularly against atrial fibrillation. Furthermore, there is a pathophysiological rationale for the investigation of ranolazine in the treatment of diastolic dysfunction and failure. However, additional data are needed before the use of ranolazine in the treatment of arrhythmias or heart failure can be recommended.

## *Management of Heart Failure*

### β-Blockers

β-blockers are recommended in addition to ACE inhibitors for the management of heart failure. A response to β-blockers therapy in heart failure has been associated with the ACE genotype. It appears that increased angiotensin II concentrations associated with the D allele may cause increased activation of the sympathetic

nervous system and that patients with the D allele may thus derive greater benefits from pharmacologic interventions to decrease sympathetic nervous system activity (e.g., β-blocker therapy).

Despite the proven efficacy of β-blockers, there are many reasons why so many patients with congestive heart failure are not treated with these medications. One important reason is concern for adverse reactions, which occur in 25–43 % of patients. Discontinuation of therapy is frequent due to hypotension, bradycardia and worsening of heart failure. This has led to the study of genetic variants that determine response to β-blockers. Polymorphisms in the gene coding for the CYP2D6 isoenzyme, which catalyzes the metabolism of β-blockers such as metoprolol, carvedilol, timolol, and propranolol, may also affect β-blocker response. It is possible that the CYP2D6-related genotype interacts with drug target polymorphisms (e.g., β-receptor polymorphisms) and polymorphisms in genes involved in pathophysiology (e.g., the ACE I/D polymorphism) to influence the overall response to β-blockers.

In addition to genetic variants that affect plasma concentrations of a drug, variants in drug target, the $\beta_1$-receptor could also alter responses to β-blockers. A clinical study of titration of metoprolol controlled release/extended release in heart failure revealed that patients with the Gly389 variant and Ser49Ser genotype of $\beta_1$-receptor are significantly more likely to require increases in heart failure medications during β-blocker titration and thus may require more frequent follow-up during titration (Terra et al. 2005).

**Bucindolol**

Bucindolol's unique pharmacology in advanced heart failure patients is to produce either a hyper-response (a β1 receptor polymorphism) or avoid an adverse effect (an α2c receptor polymorphism). These dual gene loci create a set of diplotypes characterizing the population. By identifying important genetic factors underlying heart failure and the response to bucindolol, Arca Discovery Inc has identified those patients who will benefit most from bucindolol treatment. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure. A study concluded that beta(1)AR-389 variation alters signaling in multiple models and affects the β-blocker therapeutic response in heart failure and, thus, might be used to individualize treatment of the syndrome (Liggett et al. 2006).

When prescribed genetically, bucindolol will be the state of the art in heart failure treatment for a majority of the of the US heart failure population. Bucindolol's unique pharmacology gives it other advantages as well, such as superior myocardial infarction clinical endpoints and tolerability.

**BiDil**

Enalapril therapy is associated with a significant reduction in the risk of hospitalization for heart failure among white patients with left ventricular dysfunction, but not among similar black patients. This finding underscores the need for additional

research on the efficacy of therapies for heart failure in black patients. This analysis, combined with other recent data from clinical trials, suggests that the overall population of black patients with heart failure may be underserved by current therapeutic recommendations. The fact that large-scale trials of therapy for heart failure have been performed in preponderantly white populations has limited the ability of the medical community to assess the efficacy of current therapies in black patients.

The relatively high level of heart failure in the black population has been attributed, in part, to a lack of nitric oxide (NO). BiDil (NitroMed), made of isosorbide dinitrate and hydralazine, is thought to reduce mortality in this population by restoring depleted NO levels, and by protecting NO that is formed naturally in vascular endothelial cells. A randomized trial has examined whether a fixed dose of Bidil provides additional benefit in blacks with advanced heart failure, a subgroup previously noted to have a favorable response to this therapy (Taylor et al. 2004). Hydralazine is an antioxidant and vasodilator, which means that it protects NO formed by isosorbide dinitrate and dilates blood vessels. Neither drug is indicated separately for heart failure. The addition of a fixed dose of isosorbide dinitrate plus hydralazine to standard therapy for heart failure including neurohormonal blockers was shown to be efficacious and increased survival among black patients with advanced heart failure. The study was terminated early owing to a significantly higher mortality rate in the placebo group than in the group treated with the drug combination. NitroMed Inc has submitted the African American Heart Failure Trial (A-HeFT) clinical dataset to the FDA. The product was approved by the FDA in 2005. BiDil became the first drug to be developed and marketed on the basis of a demonstrated efficacy in black subjects and could pave the way for a generation of individualized medicines for ethnic groups.

The African American Heart Failure Trial (A-HeFT) and the FDA approval of BiDil for race-specific prescription stirred the debate about the scientific and medical status of race. An analysis has been published of the factors influencing physicians' prescription of BiDil and whether exposure to the controversy has an impact on their therapeutic judgments about the drug (Maglo et al. 2014). Overall, physicians prescribe and are willing to prescribe BiDil more to black patients than to white patients. However, physicians' lack of awareness about the controversial scientific status of A-HeFT suggests the need for more efficient ways to convey scientific information about BiDil to clinicians. Furthermore, the uncertainties about the determination of clinical utility of BiDil for the individual patient raise questions about whether this specific race-based therapy is in patients' best interest.

### *Management of Hypertension*

Hypertension (HPN) is a common disorder affecting ~20 % of the US population. Care of hypertensive patients vary a lot. Ideally, individual risks must be assessed in order for the best decision to be made as to which patients with hypertension to treat and how. Assessment identifies important cardiovascular risk factors that may

warrant treatment and helps to establish the absolute benefits that patients can expect from particular treatments. The benefits of treating hypertensive patients also vary, depending on each patient's competing risks of dying from other than cardiovascular causes. For example, patients with multiple serious conditions, such as end stage Alzheimer's disease, obstructive lung disease, frequent falls, gout, and urinary incontinence, have high competing risks that may minimize or negate the benefits of treating their hypertension.

If treatment of HPN is strictly based on BP levels some patients receive too many medications and others too little. Individualized recommendations should consider multiple factors for patients' risk of heart disease, e.g. age, gender, smoking, etc. Use of medications should be guided by a patient's risk of these diseases and how much adding a new medication decreases that risk – not solely on their BP level. Those who have mild HPN but high cardiovascular risk receive a lot of benefit from treatment, but those with low overall cardiovascular risk do not. A study found that benefit-based tailored treatment was both more effective and required less antihypertensive medication than current guidelines based on treatment to achieve specific BP goals (Sussman et al. 2013). Biomarkers may help to identify patients where HPN is linked to risk of CHD. For example high serum parathyroid hormone level is related to high diastolic BP and is also a risk factor for CHD (Zhao et al. 2014). Once the decision to treat HPN has been made, an appropriate therapy should be selected.

### Adjusting Therapy of Hypertension to Fluctuations of Blood Pressure

Blood pressure is a continuous, not a static, variable. Individuals exhibiting similar clinic or home BP can differ considerably with respect to their average day and nighttime values, and sleep, responses to mental and physical stimuli, as well as seasonal variations. Several such episodes of BP fluctuations increase cardiovascular risk independent of the average of conventionally recorded BP readings. Antihypertensive drugs differ in their effects on intersession BP variability and associated risk of stroke. Optimization of personalized cardiovascular risk assessment and attempts to reduce such risk involves identification of BP variability that best estimates individual cardiovascular risk (Floras 2013). There is need for establishing "normal" and "high-risk" BP variability distributions to test the hypothesis that attenuating such variability by drug or device therapy reduces cardiovascular risk more than BP reduction per se. Results of these studies should be integrating into clinical practice.

Another implication of BP fluctuations is that dose and delivery of antihypertensive drug should be adjusted to fluctuations of BP so that short acting doses are delivered at time of peak BP that are above the values according to guidelines. It is preferable to maintaining 24 h delivery of a drug with a prolonged release preparation. Adjusted delivery is feasible with a BP sensor linked to a transdermal patch with controlled release. Although transdermal drug delivery technology has advanced enough to accomplish this, BP sensor technology needs further development to make a system that is easy to use by the patient.

### Choice of Drugs for Hypertension

Over 100 medications are available for treatment of HPN in several categories: diuretics, α-blockers, β-blockers, aldosterone antagonists, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, CNS active agents and calcium channel blockers. Each of these categories contains several distinct drugs, which vary in their efficacy and liability to produce adverse reactions in different patient populations. β-adrenergic antagonists are generally recommended as first-line therapy, along with thiazide diuretics, for the treatment of HPN. However, as many as 60 % of hypertensive patients do not achieve adequate lowering of BP from monotherapy with β-blockers. It is plausible that genetic variation in the β-adrenergic-receptor genes accounts for some of the observed variability in BP response.

Antihypertensive monotherapy does not address the multifactorial nature of HPN as a disease with many pathways. An approach to increase chances of efficacy is to use fixed combination of drugs with different modes of action as initial therapy in the treatment of HPN. The additive or synergistic effect of combination therapy may lower blood pressure in patients who tend to have less than full response to one component only. This is still an approximate method and may increase the adverse effects of drug interactions unless the combination is selected individually for each patient.

### Correction of Causes and Risk Factors of Hypertension

A number of causes and risk factors of HPN have been identified and these should be corrected. Low-salt diet for salt-sensitive HPN and management of stress by relaxation and meditation are well known and should be incorporated in personalized life style modification advice. A mendelian randomization study has shown that low plasma 25-hydroxyvitamin D (25OHD) concentration is one cause of HPN and this is partially due to genetic variants associated with low endogenous production of 25(OH)D, raising the possibility of preventing or reducing HPN with vitamin D supplementation (Vimaleswaran et al. 2014). This finding warrants further investigation in an independent, similarly powered study.

### Genes and Hypertension

Recently there is increasing interest in genes related to hypertension. Genetic factors account for 40–50 % of a person's susceptibility to hypertension. HPN is more prevalent and contributes to more severe manifestations of cardiovascular disease in African Americans than in any other US ethnic group. Previous searches of the genome found limited evidence of genes that determine HPN. So far, most gene discovery studies have involved people of European descent. A landmark study involving nearly 30,000 African-Americans has discovered four novel gene variations associated with blood pressure, which are also associated with blood pressure across other populations (Franceschini et al. 2013). Although it is unknown how the genes regulate blood pressure, the findings contribute to better understanding of

blood pressure pathways that can lead to future development of drug target for hypertension and may guide therapy for clinical care. The authors of the study are conducting additional research to determine whether the four genes respond to existing hypertension medications. Being a polygenic disorder, HPN still remains a challenge for designing better future treatments. Individuals typically respond differently to a given medication depending on which gene mutation they carry. The more information researchers gather, the greater opportunity clinicians will have to prescribe the drug that is most efficacious based on the patient's specific mutation.

### Improving Management of HPN by Targeting New Pathways

It is well known that BP is maintained by electrical impulses from the brain that travel to the arteries via the sympathetic nervous system and control their diameter, but this pathway is often chronically overactive in HPN. Most antihypertensive drugs work by decreasing both acute and chronic activity in the sympathetic nervous system. However, these drugs often have serious side effects, such as fatigue, dizziness, and erectile dysfunction. These drawbacks have led to the search for novel ways to inhibit the sympathetic nervous system while causing fewer problems for patients with HPN.

A study has found a new link between the brain and increased BP, a steroid called ouabain, as well the pathway that connects the brain to ouabain's effects on proteins that regulate arterial calcium and contraction (Hamlyn et al. 2014). The proximal components of this axis are neuronal pathways activated by brain angiotensin II (Ang II) that depend upon central aldosterone and mineralocorticoid receptors. The distal components of the axis include up-regulated circulating levels of ouabain and related steroids, and functional reprogramming of arterial function due to increased expression of arterial myocyte proteins that raise arterial myocyte $Ca^{2+}$ and myogenic tone resulting in augmentation of sympathetic responses. These results are consistent with the idea that long term increases in central Ang II and circulating ouabain sustain BP via the combined effects of heightened sympathetic activity and the functional reprogramming of arterial function. These findings suggest new approaches for treating HPN by modifying this pathway.

### Individualized Therapy of HPN Based on Risk Factors of Heart Disease

Individualized recommendations consider multiple factors for patients' risk of heart disease, e.g. age, gender, smoking, etc. Use of medications should be guided by a patient's risk of these diseases and how much adding a new medication decreases that risk – not solely on their BP level. If treatment of HPN is strictly based on BP levels some patients receive too many medications and others too little.

Those who have mild HPN but high cardiovascular risk receive a lot of benefit from treatment, but those with low overall cardiovascular risk do not. Individualized treatment of HPN could prevent >25 % of heart attacks and strokes while using less medications. Patients with HPN and hypercholesterolemia, who are treated for both concurrently, are 50 % less likely to get heart disease (Egan et al. 2014).

### Pharmacogenomics of Diuretic Drugs

Diuretics are considered to be the first-line drugs for HPN but their overall efficacy is not sufficient. Many patients suffer adverse effects such as disturbances of serum $K^+$ levels. Variations in efficacy and susceptibility to adverse reactions of diuretics may be partially caused by genetic polymorphisms of genes involved in the pharmacodynamics and pharmacokinetics of diuretics. Genes with a role in the pharmacokinetics of most diuretics are renal drug transporters, especially OAT1, OAT3 and OCT2 (genes SLC22A6, SLC22A8 and SLC22A2) whereas variants in carbonic anhydrase (CA), CYP450 enzymes and sulfotransferases are relevant only for specific substances. Genes on the pharmacodynamic side include the primary targets of thiazide, loop, $K^+$-sparing and aldosterone antagonistic diuretics: NCC, NKCC2, ENaC and the mineralocorticoid receptor (genes SLC12A3, SLC12A1, SCNN1A, B, G and NR3C2). Polymorphisms in these and in associated proteins, e.g. GNB3, α-adducin and ACE, seem to be clinically relevant.

A particular genetic alteration in hypertensive patients dramatically increases the risk of heart attack, stroke or death, and may explain why some hypertensive patients fare worse than others, even if they take the same medication. Patients carrying α-adducin gene are less likely to suffer a heart attack or stroke if they were taking a diuretic. Data from the International Verapamil SR-Trandolapril study (INVEST-GENES) suggested that one genotype group benefited from the diuretic and had a reduction in heart attack and stroke, while the other genotype group did not. In the INVEST sub study, nearly a third of the participants were carriers of the tryptophan version of the alpha-adducin gene, a protein associated with the movement of ions, especially sodium, across cells. In these individuals, the amino acid glycine has been swapped with the amino acid tryptophan. Up to 40 % of the population carries at least one copy of the tryptophan form of the gene. Patients with this version had a 43 % higher risk of heart attack, stroke or death than those with the glycine form in the 2½ years after the study began. But unlike previous research, the UF study did not show that patients with the glycine form benefited more from diuretics, which help lower blood pressure by removing excess salt and water from the body. The findings of this study may enable patients to receive appropriate personalized medicine based on their genetic makeup.

### Pharmacogenomics of ACE Inhibitors

Polymorphism of the ACE gene is known to influence the response to ACE inhibitor fosinopril in hypertensive patients. Blacks with HPN, as a group, have lower plasma renin activity and are less likely than hypertensive whites to achieve adequate blood pressure reductions with ACE inhibitor monotherapy. HPN is considered to be a good model for development of personalized medicine because it is a multifactorial disease.

It is now possible to identify a subgroup of hypertensive patients (30 %) that should be treated with ACE-inhibitors as first line treatment, since they will show a much better response than the remaining population. This test has been expanded to cover a panel of different classes of antihypertensive treatments, such as angiotensin II antagonists and β-blockers. Such a test enables the selection of the most effective

drug as first line treatment leading to reduction of the number of drugs required for adequate treatment as well the number of visits by the patient to the health-care facility for monitoring of blood pressure. The overall effect would be improvements in quality of health care and cost savings.

### Prediction of Antihypertensive Activity of Rostafuroxin

Two mechanisms, among others, are associated with essential HPN and related organ damage: mutant α-adducin variants and high concentrations of endogenous ouabain. An antihypertensive agent, rostafuroxin, selectively inhibits these mechanisms in rodents. A study has investigated the molecular and functional effects of mutant α-adducin, ouabain, and rostafuroxin in hypertensive rats, human cells, and cell-free systems and demonstrated that both mutant α-adducin variants and the ouabain–Na,K-ATPase ($Na^+$- and $K^+$-dependent adenosine triphosphatase) complex can interact with the Src-SH2 (Src homology 2) domain, increasing Src activity and the Src-dependent Na,K-ATPase phosphorylation and activity (Ferrandi et al. 2010). Wild-type α-adducin or Na,K-ATPase in the absence of ouabain showed no interaction with the Src-SH2 domain. Rostafuroxin disrupted the interactions between the Src-SH2 domain and mutant α-adducin or the ouabain–Na,K-ATPase complex and blunted Src activation and Na,K-ATPase phosphorylation, resulting in blood pressure normalization in the hypertensive rats.

The translatability of these data to humans was also shown in a pharmacogenomic clinical trial, which investigated the relationship between variants of genes encoding enzymes for ouabain synthesis (lanosterol synthase) and HSD3B1 (hydroxy-δ-5-steroid dehydrogenase, 3β- and steroid δ-isomerase 1), ouabain transport and adducin activity, and the responses to antihypertensive medications (Lanzani et al. 2010). The genetic profile defined by these variants predicted the antihypertensive effect of rostafuroxin, a sodium pump blocker, but not that of losartan or hydrochlorothiazide. The magnitude of the rostafuroxin antihypertensive effect was twice that of antihypertensive drugs recently tested in phase II clinical trials. One-quarter of patients with primary HPN display these variants of adducin or concentrations of endogenous ouabain and would be expected to respond to therapy with rostafuroxin. Because the mechanisms that are inhibited by rostafuroxin also underlie HPN-related organ damage, this drug may also reduce the cardiovascular risk in these patients beyond that expected by the reduction in systolic blood pressure alone. The results open up the possibility of improved patient stratification, as they allow predictions to be made about the effectiveness of rostafuroxin (but not that of any other antihypertensive drugs) in patients carrying key gene variants.

### Role of Pharmacogenetics in Management of Hypertension

Genetic factors may influence the response to antihypertensive medication. A number of studies have investigated genetic polymorphisms as determinants of cardiovascular response to antihypertensive drug therapy. Hypertensive patients with the 460 W allele of the α-adducin gene have a lower risk of myocardial infarction and

stroke when treated with diuretics compared with other antihypertensive therapies. With regard to BP response, interactions were also found between genetic polymorphisms for eNOS and diuretics and the ACE gene and angiotensin II type 1 receptor antagonists. Although there are controversies to settle and difficulties to overcome, pharmacogenetics may yield successful strategies to optimize drug therapy. Several candidate genes are currently under investigation for their potential to modify response to antihypertensive drugs. Findings from previous studies require conformation in other studies to be able to make definitive conclusions about current positive drug-gene interactions. It is also important that research groups collaborate more in order to facilitate the conduct of a metaanalysis for conclusive results. With the development of efficient methods for analyzing massive amounts of data, pharmacogenetic studies may eventually lead to the optimization of antihypertensive drug therapy based on genetic profiles of patients.

Pharmacogenetic-guided therapy has clinical potential for management of HPN, but there are few controlled studies on this topic. A clinical trial on individuals with uncomplicated HPN aims to identify the genetic determinants of the antihypertensive and adverse metabolic responses to a thiazide diuretic (hydrochlorothiazide), a β-blocker (atenolol), and their combination (Johnson et al. 2009). This will be accomplished through candidate gene and genome-wide association approaches. Current antihypertensive therapy is discontinued, and HPN is confirmed, along with collection of other baseline data. Subjects are then randomized to either hydrochlorothiazide or atenolol, with one dose titration step, followed by assessment of response to therapy after at least 6 weeks on the target dose. Those with blood pressure >120/70 mmHg have the second drug added, with similar dose titration and response assessment procedures. Data collected include home, office, and 24 h ambulatory blood pressure. Biological samples collected in the fasting state include plasma, serum, DNA, and urine. This trial will add substantially to our understanding of the genetic determinants of antihypertensive and adverse metabolic responses to two commonly used antihypertensive drug classes.

#### Scheme for Management of Hypertension by Personalized Approach

Despite the many therapeutic options for HPN, only 27 % of the patients achieve adequate control of BP. Therefore, there is an opportunity to improve the management of HPN by a personalized approach as shown in Fig. 14.1.

## *Pharmacogenetics of Lipid-Lowering Therapies*

Cardiovascular disease is associated with nonmodifiable risk factors such as age, gender, and genetic background, and with modifiable risk factors such as lipid concentrations. Lowering serum lipid levels has been demonstrated to slow the progression of, or even induce regression in, atherosclerosis. However, like any other

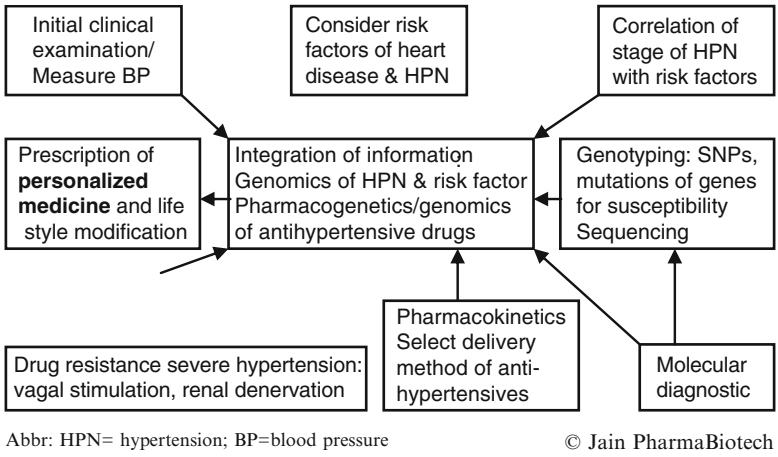


**Fig. 14.1** A scheme of personalized approach to management of hypertension

drug treatment, the magnitude of plasma lipid responses to drug therapies varies considerably among individuals modified by a number of factors such as age, gender, concomitant disease and genetic determination. Pharmacogenetics provides the experimental basis to understand the variability in response to drugs as a function of the individual genetic makeup. Information from small clinical trials reveals that several candidate genes may hold some promise in our quest to predict individual success to hypolipidemic drug treatment.

### Polymorphisms in Genes Involved in Cholesterol Metabolism

Polymorphisms in genes involved in cholesterol synthesis, absorption, and transport may affect statin efficacy. Genetic variation at the LDL receptor locus can affect baseline lipids, response to pravastatin, and cardiovascular disease risk in subjects placed on statin treatment (Polisecki et al. 2008). The DNA of 1,536 individuals treated with pravastatin, was analyzed for 148 SNPs within 10 candidate genes related to lipid metabolism (Chasman et al. 2004). Variation within these genes was then examined for associations with changes in lipid levels observed with pravastatin therapy. Two common and tightly linked SNPs were significantly associated with reduced efficacy of pravastatin therapy. Both of these SNPs were in the gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the target enzyme that is inhibited by pravastatin. The association for total cholesterol reduction persisted even after adjusting for multiple tests on all 33 SNPs evaluated in the HMG-CoA reductase gene as well as for all 148 SNPs evaluated was similar in magnitude and direction among men and women and was present in the ethnically diverse total cohort as well as in the majority subgroup of white participants. Thus, individuals heterozygous for a genetic variant in the HMG-CoA

reductase gene may experience significantly smaller reductions in cholesterol when treated with pravastatin. The absolute difference in total cholesterol reduction associated with HMG-CoA reductase was significant enough to affect health outcome. Future studies should determine if this difference can be offset by adjustment of dose or use of a non-statin cholesterol-lowering agent.

There is interindividual variation in low-density lipoprotein cholesterol (LDLc) lowering by statins. An intronic SNP in ABCA1 and the APOE ε3 allele are associated with reduced LDLc lowering by statins and identify individuals who may be resistant to maximal LDLc lowering by statins (Voora et al. 2008).

HMG-CoA reductase inhibitors are generally very well tolerated but there are two uncommon but potentially serious adverse effects related to HMG-CoA reductase inhibitor therapy: hepatotoxicity and myopathy. The occurrence of lethal rhabdomyolysis in patients treated with cerivastatin has prompted concern on the part of physicians and patients regarding the tolerability of HMG-CoA reductase inhibitors. CYP2D6 plays an important role in the metabolism of simvastatin. It has been shown that the cholesterol-lowering effect as well as the efficacy and tolerability of simvastatin are influenced by CYP2D6 genetic polymorphism. Because the different HMG-CoA reductase inhibitors differ, with respect to the degree of metabolism by the different CYP enzymes, genotyping may help to select the appropriate HMG-CoA reductase inhibitor and the optimal dosage during the start of the treatment and will allow for more efficient individual therapy.

### Role of eNOS Gene Polymorphisms

The eNOS gene harbors a common polymorphism in intron 4 (4a/b), and some clinical studies have suggested an association of the rare a-allele with coronary artery disease and myocardial infarction. However, contradictory results have also been reported. One study has investigated the associations of eNOS polymorphism with these diseases in two prospective autopsy series. In one, no significant differences in areas of atherosclerotic lesions and coronary stenosis percentages were found between men carrying the a-allele (ba + aa) compared with those homozygous for the b-allele. Subjects with the a-allele had significantly lower risk of myocardial infarction compared with those carrying the bb genotype. Men with the a-allele also tended to have coronary thrombosis less often. The eNOS gene 4a/b polymorphism was not associated with the extent of coronary atherosclerosis, but the a-allele of the variant seems to protect to some degree against the development of myocardial infarction. In the second, a placebo-controlled study, adenosine-stimulated myocardial perfusion, as determined by PET, improved after treatment with pravastatin in subjects with the eNOS ba-genotype but not in those with the bb-genotype. This effect is not dependent on the decrease of serum cholesterol.

However, the current clinical relevance of this knowledge is quite limited due to the small effects observed for each of the genetic markers examined. Future progress in this area will be driven by studying gene-gene and gene-treatment interactions in much larger patient populations.

### Prediction of Response to Statins

Statins are the most frequently prescribed drugs for lowering LDL-cholesterol (LDLC) levels and risk of cardiovascular disease. Statins reduce LDLC levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis. Although numerous clinical trials have demonstrated efficacy of statins, there is substantial inter-individual variation in the magnitude of statin-induced LDLC reduction. To date, analysis of individual DNA sequence variants has explained only a small proportion of this variability.

Marked lowering of LDLC levels (< or = 50 %) with intensive statin therapy is associated with major reduction in cardiovascular risk, but it is limited by a potential increase in adverse effects, thereby justifying optimization of LDL-C reduction with minimal risk. The organic anion transporting polypeptide-1B1 encoded by the SLCO1B1 gene is implicated as a major transporter in cellular uptake of statins, and notably fluvastatin. Results of a pharmacogenomics study on elderly subjects with hypercholesterolemia reveal that OATP1B1 gene is implicated in the pharmacological action and efficacy of fluvastatin (Couvert et al. 2008). The common *14 allele of SLCO1B1, which is distinguished by the presence of the c.463C > A polymorphism, was associated with enhanced lipid-lowering efficacy in this study.

Transcriptomic analyses have been used to identify additional genetic contributions to inter-individual differences in statin efficacy. Using expression array data from immortalized lymphoblastoid cell lines derived from participants in the Cholesterol and Pharmacogenetics clinical trial, investigators identified 100 signature genes differentiating high versus low statin responders (Kim et al. 2014). Two of the signature genes, CYP51A1 and NFYC, have been previously implicated in cholesterol metabolism. The enzyme encoded by CYP51A1, lanosterol 14-α-demethylase, catalyzes the conversion of lanosterol to 24,25-dihydrolanosterol in one of the later steps of the cholesterol biosynthesis pathway. A radial-basis support vector machine prediction model of these signature genes with addition of SNPs previously reported to be associated with statin response in GWAS results in a combined model that predicts 15 % of the most extreme 15 % of high and low responders with high accuracy. These results demonstrate that transcriptomic information, combined with genetic information, is a substantial contribution to variance of LDLC response to statin treatment. This may provide a framework for identifying novel pathways that influence cholesterol metabolism.

### Personalized Management of Women with Hyperlipidemia

Several studies have shown that C-reactive protein (CRP) levels may be more important than cholesterol levels for predicting cardiovascular events such as heart attacks. In particular, these studies have shown that elevated CRP is a risk factor that is independent of cholesterol levels. It had previously been shown that HRT caused elevated levels of CRP and of heart attacks and strokes (Women's Health Initiative).

The protective effect of a key genetic variant may be overwhelmed by the use of hormone replacement therapy. The results of these studies give lifestyle guidance for women who would like to preserve the protective benefits conferred by favorable genetic variations, and may ultimately lead to new or modified drugs. Men and women with common variants in the apolipoprotein E (APOE) gene on average have naturally lower levels of CRP. In the case of women, however, this beneficial effect may be largely neutralized by HRT, allowing CRP levels to potentially increase to dangerous levels.

### Therapeutic Alternatives in Patients with Statin Intolerance

A significant number of patients are unable to tolerate statins due to side effects, including:

- Myopathy: muscle pain or weakness or even muscle breakdown.
- Increased glucose levels and increased the risk of worsening of glycemic control and of new onset diabetes.

ETC-1002 (Esperion Therapeutics Inc) is an orally available small molecule designed to lower levels of LDL-C and to avoid side effects associated with existing LDL-C lowering therapies. ETC-1002 has a unique dual mechanism of action that has the potential to regulate both lipid and carbohydrate metabolism. ETC-1002 appears to work by inhibiting ATP citrate lyase, a key enzyme in the cholesterol biosynthetic pathway, and activating a complementary enzyme, 5′-adenosine monophosphate-activated protein kinase. Both enzymes are known to play significant roles in the synthesis of cholesterol and glucose in the liver. By inhibiting cholesterol synthesis in the liver, ETC-1002 causes the liver to take up LDL particles from the blood, which reduces LDL-C levels. It has been studies in phase I and II clinical trials.

## *Thrombotic Disorders*

A number of thrombotic disorders cause cardiovascular disease. Venous thrombosis has an annual incidence of 1 per 1,000 in the general population and is associated with significant morbidity and mortality. Several genetic variants have been identified that are associated with an increased risk of venous thrombosis, including a recently discovered mutation in the prothrombin gene. Factor V Leiden mutation is associated with 15–20 % of the cases of idiopathic thrombotic disorders.

### Factor V Leiden Mutation

A mutation in the procoagulant protein Factor V (Factor V Leiden) causes it to be relatively resistant to degradation by activated protein C (APC), resulting in a thrombotic tendency. The mutation is a guanine-to-adenine substitution at nucleotide 1651 that results in a glutamine-to-arginine substitution at position 506 (R506Q).

This is a clinically significant mutation, since it is relatively common (found in 3–6 % of Caucasian subjects) and has been shown to be associated with venous thrombosis and stroke. It is of special importance in women for the following reasons:

- It increases the risk of venous thrombosis associated with oral contraceptives and hormone replacement therapy.
- It synergizes with pregnancy which, by itself, increases the risk of venous thrombosis
- It is associated with intrauterine growth restriction, still births and cerebral palsy in the off-spring
- It is associated with myocardial infarction in young women but not in young men

This mutation can be readily detected by molecular diagnostics. The presence of Factor V mutation is an important consideration for anticoagulant therapy to prevent thromboembolism and should be individualized for each patient. CYP2C9 mutation is a predicator for anticoagulation-related in these patients.

**Anticoagulant Therapy**

Warfarin is widely used to prevent thromboembolic events in patients with atrial fibrillation, prosthetic heart valves, and previous cerebrovascular events. Warfarin is a narrow-therapeutic-index drug; inadequate or excessive anticoagulation may result in substantial morbidity and potentially in death because of thromboembolic complications or bleeding. Warfarin therapy is complicated by great interpatient variability in the dosage needed to achieve optimal anticoagulation.

Several genes play a role in warfarin's metabolism. The S-isomer of warfarin has five times the anticoagulant activity of the R-isomer and is metabolized by CYP2C9. Polymorphisms in CYP2C9, a gene for cytochrome P450, cause about 30 % of patients to be slow warfarin metabolizers, which could result in high blood concentrations. Testing for CYP2C9 polymorphisms provides a better starting point for the warfarin dose, which would achieve stable blood levels more quickly than trial-and-error dosing. Many Caucasians (~50 %) possess less active forms of CYP2C9, a key enzyme in warfarin metabolism: 10-fold interpatient variability in the dose of warfarin required to attain a therapeutic response. Frequent assessment of anticoagulation status is necessary during warfarin therapy to ensure drug efficacy and to prevent or minimize hemorrhagic events. Thus, the identification of factors that influence warfarin dosage requirements would be of great benefit in the management of patients at risk for coagulation disorders. Polymorphisms in the vitamin K epoxide reductase multiprotein complex (VKOR) also affect warfarin metabolism in rats. Mutations in one of the complex's subunits, VKORC1, confer warfarin resistance in some human disorders. Genotyping for both CYP2C9 and VKORC1 should be considered when prescribing warfarin before surgery.

Heparin is used to prevent and treat thromboembolic diseases. One of the most serious adverse reactions to heparin is immune-related heparin-induced thrombocytopenia (HIT), which can result in severe thromboembolic complications and death.

Heparin-induced antibodies recognize and bind to heparin-platelet factor 4 complexes and subsequently activate platelets via the platelet Fc -receptor to mediate HIT. A SNP commonly occurs in the platelet Fc -receptor gene, resulting in an arginine or histidine at codon 131 (131Arg/His), and appears to affect platelet aggregation.

Invasive procedures on patients receiving anticoagulation therapy require an individualized assessment of the risk of bleeding versus the risk of thrombo-embolic events. Besides SNPs, tailoring anticoagulation therapy according to the risk of individual patients is the best way to optimize the benefit/risk ratio, and is recommended in treatment guidelines of fondaparinux (a heparin analog) as well as dabigatran etexilate, the oral direct thrombin inhibitor (Rosencher and Albaladejo 2012). Dose of fondaparinux should be reduced in renal impairment. Availability of two approved doses of dabigatran etexilate for thromboprophylaxis following orthopedic surgery enables the dose to be tailored to the individual patient's characteristics, based on the age and renal function of the patient, as recommended by the European Medicines Agency, in order to maintain efficacy while decreasing bleeding risk.

A multicenter, randomized, controlled trial of warfarin involving patients with atrial fibrillation or venous thromboembolism in whom genotyping for CYP2C9*2, CYP2C9*3, and VKORC1 (−1639G → A) was performed with the use of a POC test (Pirmohamed et al. 2013). Results showed that pharmacogenetic-based dosing was associated with a higher percentage of time in the therapeutic INR range than was standard dosing during the initiation of warfarin therapy. Acenocoumarol (Sintrom or Sinthrome), a derivative of coumarin, is an anticoagulant that functions as a vitamin K antagonist like warfarin. Three genetic polymorphisms involving the genes VKORC1, CYP2C9 and CYP4F2 are considered to be useful for establishing the correct dosage. However, two single-blind randomized clinical trials, comparing a genotype-guided dosing algorithm based on clinical variables and genotyping for CYP2C9 and VKORC1 with a dosing algorithm that included only clinical variables showed that genotype-guided dosing of acenocoumarol or phenprocoumon did not improve the percentage of time in the therapeutic INR range during the 12 weeks after the initiation of therapy (Verhoef et al. 2013). An editorial comment on pharmacogenomic guided anticoagulation commented that we should concentrate on improvements in the infrastructure for INR testing, including better communication among the laboratory, the physician, and the patient; in the use of formal algorithms for dosing, without concern for genotype; in patient adherence to therapy and possibly more responsibility for dosing being assumed by the patient; and in increased diligence by medical and paramedical personnel in testing, monitoring, and dosing on the basis of the INR, given the high percentage of medical mismanagement associated with these anticoagulant agents (Furie 2013).

### Antiplatelet Therapy

Prasugrel, an approved antiplatelet drug for cardiovascular thrombotic disease, is a prodrug with rapid and almost complete absorption after oral ingestion of a loading dose. It is metabolized into its active form, which binds irreversibly to the adenosine

diphosphate (ADP) P2Y12 receptor on platelets for their lifespan, thereby inhibiting their activation and decreasing subsequent platelet aggregation. Hydrolysis by intestinal carboxylesterases and oxidation by intestinal and hepatic cytochrome P-450 enzymes convert prasugrel into its active metabolite. Prasugrel has a greater antiplatelet effect than clopidogrel because it is metabolized more efficiently. Genetic polymorphisms affecting the cytochrome P450 system may explain some of the differences in metabolism between prasugrel and clopidogrel.

Resistance to clopidogrel, an antiplatelet therapy, has been shown to be present in 25–30 % of Caucasians and an even higher percentage in Asians. Part of this resistance is because of the CYP2C19*2 allele. CYP2C19 variant alleles are independent predictors of clopidogrel response variability and occurrence of major adverse cardiovascular events in high-risk vascular patients on clopidogrel therapy. Increasing evidence suggests a combination of platelet function testing with CYP2C19 genetic testing may be more effective in identifying high-risk individuals for alternative antiplatelet therapeutic strategies. A crucial point in evaluating the use of these polymorphisms in clinical practice, besides test accuracy, is the cost of the genetic test and rapid availability of the results. One study has genotyped 100 acute coronary syndrome patients for CYP2C19*2,*3,*4,*5, and *17 polymorphisms with two platforms: Verigene® and the TaqMan® system (Saracini et al. 2012). Genotyping results obtained by the classical TaqMan approach and the rapid Verigene approach showed a 100 % concordance for all the five polymorphisms investigated. The Verigene system had shorter turnaround time with respect to TaqMan. The cost of reagents for TaqMan genotyping was lower than that for the Verigene system, but the effective staff involvement and the relative cost resulted in higher cost for TaqMan than for Verigene. In conclusion, Verigene system demonstrated good performance in terms of turnaround time and cost for the evaluation of the clopidogrel poor metabolizer status, giving genetic information in suitable time (<2.5 h) for a therapeutic strategy decision.

Genetic testing for aspirin resistance is not yet recommended because of incomplete genetic data. Studies to determine the value of genetic testing before the administration of warfarin are ongoing. Testing for SLCO1B1 allele in individuals with muscle cramps who are taking statins could be very helpful but is not yet recommended as a routine.

## *Nanobiotechnology-Based Personalized Therapy of Cardiovascular Diseases*

The future of cardiovascular diagnosis already is being impacted by nanosystems that can both diagnose pathology and treat it with targeted delivery systems. The potential dual use of nanoparticles for both imaging and site-targeted delivery of therapeutic agents to cardiovascular disease offers great promise for individualizing therapeutics. Image-based therapeutics with site-selective agents should enable verification that the drug is reaching the intended target and a molecular effect is occurring. Experimental studies have shown that binding of paclitaxel to smooth muscle

cells in culture has no effect in altering the growth characteristics of the cells. If paclitaxel-loaded nanoparticles are applied to the cells, however, specific binding elicits a substantial reduction in smooth muscle cell proliferation, indicating that selective targeting may be a requirement for effective drug delivery for in this situation. Similar behavior has been demonstrated for doxorubicin containing particles. Intravenous delivery of fumagillin (an antiangiogenic agent)-loaded nanoparticles targeted to αvβ3-integrin epitopes on vasa vasorum in growing plaques results in marked inhibition of plaque angiogenesis in cholesterol fed rabbits. The unique mechanism of drug delivery for highly lipophilic agents such as paclitaxel contained within emulsions depends on close apposition between the nanoparticle carrier and the targeted cell membrane and has been described as "contact facilitated drug delivery." In contrast to liposomal drug delivery (generally requiring endocytosis), the mechanism of drug transport in this case involves lipid exchange or lipid mixing between the emulsion vesicle and the targeted cell membrane, which depends on the extent and frequency of contact between two lipidic surfaces. The rate of lipid exchange and drug delivery can be greatly increased by the application of clinically safe levels of ultrasound energy that increase the propensity for fusion or enhanced contact between the nanoparticles and the targeted cell membrane.

The combination of targeted drug delivery and molecular imaging with MRI has the potential to enable serial characterization of the molecular epitope expression based on imaging readouts. Monitoring and confirmation of therapeutic efficacy of the therapeutic agents at the targeted site would permit personalized medical regimens.

## *Project euHeart for Personalized Management of Heart Disease*

In 2008, the European Union (EU) funded a research project called 'euHeart', which is aimed at improving the diagnosis, therapy planning and treatment of cardiovascular disease. It was completed in 2012 and the final results were published on web site: http://www.euheart.eu. The project combined 16 industrial, clinical and academic partners, whose collective goal was the development of individualized, computer-based, human heart models. Led by Philips Healthcare, euHeart aimed to develop advanced computer models of the human heart that can be personalized to patient-specific conditions using clinical data from various sources, such as CT and MRI scans, measurements of blood flow and blood pressure in the coronary arteries and ECGs. These computer models integrated the behavior of the heart and the aorta at molecular, cellular, tissue and organ-level. They also incorporated clinical knowledge about how cardiovascular disease disturbs the correct functioning of the heart at these levels. Atrial modeling is currently in a transition from the sole use in basic research to future clinical applications (Krueger et al. 2013).

euHeart significantly advanced the state-of the-art in cardiac simulations. The project demonstrated the predictive value and clinical potential of personalized cardiac simulations for several clinically relevant settings. As a result, it may be possible to develop simulation tools that physicians can use to predict the outcome of different

types of therapy, and because the models will be personalized to individual patients, the therapy could be equally personalized. The technology can modify treatment of heart rhythm disorders by a minimally invasive procedure known as radio-frequency ablation. During this procedure, a catheter is inserted into the patient's heart and the tissue responsible for propagating abnormal electrical signals through the heart muscle is destroyed using heat from a radio-frequency field generated at the tip of the catheter. Currently, physicians have to rely on their experience to decide which areas of tissue to destroy – a task that is complicated by the fact that the electrical activity in every patient's heart is subtly different. With the aid of a computerized model that reflects the patient's unique heart structure and function, it may be possible to test the results of destroying different areas of tissue before operating on the patient.

### *Concluding Remarks on Personalized Management of Cardiovascular Diseases*

Individual responses to drugs vary and are partly determined by genes. Simple genetic analyses can improve response prediction and minimize side effects in cases such as warfarin and high doses of simvastatin. NGS will facilitate the identification of mutations causing cardiovascular diseases. In contrast to monogenic diseases genetic testing plays no practical role yet in the management of multifactorial cardiovascular diseases. Cell culture models based on iPSCs open the perspective of individualized testing of cardiovascular disease severity and pharmacological or genetic therapy. Biomarkers can identify individuals with increased cardiovascular risk and biomarker-guided therapy represents an attractive option with troponin-guided therapy of acute coronary syndromes as a successful example (Eschenhagen and Blankenberg 2013). Personalized approaches will gain increasing importance in the management of cardiovascular diseases in the future.

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# Chapter 15
# Personalized Management of Pulmonary Disorders

## Introduction

There are a large number of pulmonary disorders some of which present challenges in management. Role of genetic ancestry in lung function is under investigation. There is still limited information on pharmacogenomics and pharmacogenetics of pulmonary therapeutics. Personalized approaches to some pulmonary diseases will be described briefly as examples in this chapter.

### *Role of Genetic Ancestory in Lung Function*

A study shows that incorporating measures of individual genetic ancestry into normative equations of lung function in persons who identify themselves as African Americans may provide more accurate predictions than formulas based on self-reported ancestry alone (Kumar et al. 2010). The same argument may apply to other ancestrally defined groups; further studies in this area are necessary. Further studies are also needed to determine whether estimates informed by genetic ancestry are associated with health outcomes. The authors noted that environmental factors such as premature birth, prenatal nutrition, and socioeconomic status may also play an important role in the association between lung function and ancestry. It remains to be seen whether differences associated with race or ethnic group in the response to medications that control asthma are more tightly associated with estimates of ancestry. Although measures of individual genetic ancestry may foster the development of personalized medicine, large clinical trials and cohort studies that include assessments of genetic ancestry are needed to determine whether measures of ancestry are more useful clinically than a reliance on self-identified race.

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## Biomarkers of Pulmonary Disorders

Some of the biomarkers of pulmonary disorders in general are similar to those found in involvement of other organs. Biomarkers of pulmonary disorders with exception of lung cancer are listed in Table 15.1.

**Table 15.1** Biomarkers of pulmonary diseases

| Biomarkers | Sample | Applications |
|---|---|---|
| Alpha1-antitrypsin/AAT gene polymorphism | Blood: finger prick | Detection of AAT deficiency predisposing to emphysema |
| Angiogenic growth factor overexpression | Bronchoalveolar lavage fluid | Overexpression of VEGF and PIGF are biomarkers of chronic obstructive pulmonary disease (COPD) |
| Brain natriuretic peptide (BNP) | Plasma | Detection of pulmonary hypertension in patients with chronic lung disease |
| Calprotectin | Sputum and serum | Track changes in lung inflammation during an exacerbation of cystic fibrosis |
| CF-specific serum proteomic signature | Plasma | Cystic fibrosis (CF) |
| Chromagranin A (CgA) | Serum | A neuroendocrine activity biomarker that is increased in male smokers with impaired lung function |
| Copeptin, the precursor of vasopressin | Serum | A prognostic biomarker for poor prognosis in exacerbation of COPD requiring hospitalization |
| C-reactive protein (CRP) | Serum | Elevated in acute exacerbtion of COPD |
| $H_2O_2$ | Exhaled breath condensate | Measurement of oxidative stress in pulmonary diseases |
| F2-isoprostanes | | |
| Malondialdehyde | | |
| 4-hydroxy-2-nonenal Antioxidants | | |
| IgE level | Serum | The dose of omalizumab is that required is to reduce circulating free IgE levels to less than 10 IU per milliliter |
| Inflammation | Blood | WBC count, CRP and VCAM-1 relate to poorer lung function in the elderly |
| Nitric oxide (NO) | Exhaled breath | Inflammatory lung disorders, e.g., asthma Rhinosinusitis |
| | Urine | Higher levels of urinary NO are strongly associated with improved survival in acute respiratory distress syndrome |
| Osteoprotegerin (OPG) | Serum | Increased specifically in COPD |
| Serum amyloid A (SAA) | Serum | Exacerbtion of COPD by respiratory tract infections. |
| Surfactant proteins: | Tracheal aspirates | Interstitial lung disease |
| A (SP-A) | Bronchoalveolar lavage | Acute respiratory distress syndrome |
| D (SP-D) | Pleural effusions | Radiation pneumonitis |

## *Biomarkers of Inflammation and Lung Function in the Elderly*

Low lung function is associated with increased morbidity and mortality. It is therefore of interest to identify biomarkers that are associated with impaired lung function. Lung function (FEV1 and FVC) and a panel of 15 inflammatory biomarkers (including cytokines, chemokines, adhesion molecules, CRP and WBC count) from blood samples were analyzed subjects aged 70 years (Kuhlmann et al. 2013). WBC count, CRP and VCAM-1 were found to relate to poorer lung function. A dose-related association was found for the combination WBC count and CRP towards FEV1 and WBC and VCAM-1 towards FVC. This indicates that combination of two biomarkers yielded more information than assessing them one by one when analyzing the association between systemic inflammation and lung function.

## *Biomarkers of Oxidative Stress in Lung Diseases*

Oxidative stress is the hallmark of various chronic inflammatory lung diseases. Increased concentrations of ROS in the lungs of such patients are reflected by elevated concentrations of oxidative stress markers in the breath, airways, lung tissue and blood. Traditionally, the measurement of these biomarkers has involved invasive procedures to procure the samples or to examine the affected compartments, to the patient's discomfort. Non-invasive approaches to measure oxidative stress have been investigated. The collection of exhaled breath condensate (EBC) is a non-invasive sampling method for real-time analysis and evaluation of oxidative stress biomarkers in the lower respiratory tract airways. The biomarkers of oxidative stress such as $H_2O_2$, F2-isoprostanes, malondialdehyde, 4-hydroxy-2-nonenal, antioxidants, glutathione and nitrosative stress such as nitrate/nitrite and nitrosated species can be measured in EBC. Oxidative stress biomarkers also have been measured for various antioxidants in disease prognosis. EBC is currently used as a research and diagnostic tool in free radical research, yielding information on redox disturbance and the degree and type of inflammation in the lung. It is expected that EBC can be exploited to detect specific levels of biomarkers and monitor disease severity in response to treatment.

## *Biomarkers of Community-Acquired Pneumonia*

Community-acquired pneumonia (CAP) is one of the most common reasons for emergency department. Despite its prevalence, there are many challenges to proper diagnosis and management of pneumonia. There is no accurate and timely gold standard to differentiate bacterial from viral disease, and there are limitations in precise risk stratification of patients to ensure appropriate site-of-care decisions. Clinical risk scores such as pneumonia severity index (PSI) and CURB-65 (confusion, urea, respiratory rate, blood pressure, age >65 years), and blood biomarkers

of different physiopathological pathways are used in predicting long-term survival in patients with CAP. In a prospective study, patients admitted with CAP were followed for 6 years and Cox regression models as well as area under the receiver operating characteristics curve (AUC) were used to investigate associations between initial risk assessment and all-cause mortality (Alan et al. 2014). Initial PSI and CURB-65 scores both had excellent long-term prognostic accuracy, with a step-wise increase in mortality per risk class. The addition of inflammatory (pro-adrenomedullin) and cardiac (pro-atrial natriuretic peptide) blood biomarkers measured upon hospital admission further improved the prognostic capabilities of the PSI.

### BNP as a Biomarker of Chronic Pulmonary Disease

Circulating BNP levels were evaluated as a parameter for the presence and severity of pulmonary hypertension (PH) in patients with follow-up of ~1 y, significant pulmonary hypertension (mean pulmonary artery pressure >35 mmHg) was diagnosed in more than one-fourth of patients and led to decreased exercise tolerance and life expectancy. Elevated BNP concentrations identified significant pulmonary hypertension with a sensitivity of 0.85 and specificity of 0.88 and predicted mortality. Moreover, BNP served as a risk factor of death independent of lung functional impairment or hypoxemia. It is concluded that plasma BNP facilitates noninvasive detection of significant PH with high accuracy and can be used as a screening test for the presence of PH. In addition, BNP enables an assessment of the relevance of PH and could serve as a useful prognostic parameter in chronic lung disease.

### Plasma Biomarkers Related to Inflammation

Plasma biomarkers related to inflammation – IL-8 and enhanced neutrophil recruitment to the lung (ICAM-1) – are independently associated with increased mortality in patients with acute lung injury (ALI). Higher levels of IL-8 and ICAM-1 independently predicted death (McClintock et al. 2008). In addition, lower levels of the coagulation marker protein C were independently associated with an increased risk of death. The association of lower protein C levels with non-survivors continues to support the role for disordered coagulation in ALI/ARDS. These associations exist despite consistent use of lung protective ventilation and persist even when controlling for clinical factors that also impact upon outcomes. The two biomarkers with an independent association with mortality, IL-8 and ICAM-1, need to be studied further for their potential value in stratifying patients in clinical trials.

### Urinary NO as Biomarker

Acute respiratory distress syndrome (ARDS) is the rapid onset of respiratory failure – the inability to adequately oxygenate the blood – that often occurs in the critically ill. ALI precedes ARDS as severe respiratory illnesses progress. Both conditions can be life-threatening. In a large-scale, multicenter trial of patients with

ARDS or ALI, higher levels of nitric oxide (NO) in urine were strongly associated with improved survival, more ventilator-free days, and decreased rates of organ failure (McClintock et al. 2007). The authors speculated that NO has a beneficial effect on ALI since it scavenges oxygen free radicals that are generated during oxidative stress. Since NO increases microcirculation, it helps to better perfuse tissue beds in the lungs. The investigators offered an alternative hypothesis to explain their findings: NO created inside the body may have a beneficial effect on organs other than the lung during ALI. It might help prevent further tissue damage by improving oxygen and nutrient delivery to the tissues, while helping to decrease the amount of toxic oxygen species. The authors also speculated that NO might have antibacterial effects that could be important in infectious conditions that predispose patients to ALI.

## Personalized Therapy of Asthma

Asthma affects 5–7 % of the population of North America and may affect more than 150 million persons worldwide. Airway hyperresponsiveness (AHR) is the main feature of asthma and is defined as an increase in the ease and degree of airway narrowing in response to bronchoconstrictor stimuli. It is a chronic inflammatory disease but there is no clear definition of the disease and no single symptom, physical finding or laboratory test is diagnostic of this condition. The disease is manifested as variable airflow obstruction and recurrent bouts of respiratory symptoms. Allergans and viral infections induce an increased sensitivity. Little is known about the mechanisms that determine asthma development and severity and why some individuals have mild symptoms and require medication only when symptomatic whereas others have continuous symptoms despite high doses of several medications (refractory asthma). Asthma is often triggered by an allergic response and the environmental factors play an important role in manifestations of the disease. Although there is a significant hereditary component, genetic studies have been difficult to perform and results have been difficult to interpret. Only a few therapeutic agents based on novel mechanisms of action have been developed over the past two decades. Asthma is a complex disease with marked heterogeneity in the clinical course and in the response to treatment. Variability in the type of airway inflammation may underlie this heterogeneity. Despite treatment with inhaled glucocorticoids, many patients continue to have uncontrolled asthma that requires more intensive therapy. Approximately one in three patients with asthma who use inhaled glucocorticoids may not benefit from this therapy. Biomarkers and some of the other methods for guiding therapy of asthma are described here.

### *Biomarkers of Asthma*

Although the aim of management of patients with asthma is to control their symptoms and prevent exacerbations and morbidity of the disease, optimal management may require assessment and monitoring of biomarkers, i.e., objective measures of lung dysfunction and inflammation.

### Biomarker for Rhinovirus-Induced Asthma Exacerbation

Clinical observations suggest that rhinovirus infection induces a specific inflammatory response in predisposed individuals that results in worsened asthmatic symptoms and increased airway inflammation. A study has shown that IFN-γ-induced protein (IP)-10 is specifically released in acute virus-induced asthma, and can be measured in the serum to predict a viral trigger of acute exacerbations (Wark et al. 2007). Primary bronchial epithelial cell models of rhinovirus infection were used to identify mediators of rhinovirus infection and responded to infection with rhinovirus-16 by releasing high levels of IP-10, RANTES, and IL-16, as well as smaller amounts of IL-8 and TNF-α. IP-10, perhaps in combination with TNF-α, might be a useful clinical marker to identify rhinovirus and other virus-induced acute asthma. Additional findings suggest that IP-10 or CXCR3 (an IP-10 receptor that is highly expressed in activated T cells) might have a role in worsening of airflow obstruction and airway inflammation, and may therefore be potential therapeutic targets.

### Biomarkers for Predicting Response to Corticosteroid Therapy

International guidelines on the management of asthma support the early introduction of corticosteroids to control symptoms and to improve lung function by reducing airway inflammation. However, not all individuals respond to corticosteroids to the same extent and it would be a desirable to be able to predict the response to corticosteroid treatment. Several biomarkers have been assessed following treatment with corticosteroids including measures of lung function, peripheral blood and sputum indices of inflammation, exhaled gases and breath condensates. The most widely examined measures in predicting a response to corticosteroids are airway hyperresponsiveness, exhaled NO (eNO) and induced sputum. Of these, sputum eosinophilia has been demonstrated to be the best predictor of a short-term response to corticosteroids. More importantly, directing treatment at normalizing the sputum eosinophil count can substantially reduce severe exacerbations. The widespread utilization of sputum induction is hampered because the procedure is relatively labor intensive. The measurement of eNO is simpler, but incorporating the assessment of NO in an asthma management strategy has not led to a reduction in exacerbation rates. The challenge now is to either simplify the measurement of a sputum eosinophilia or to identify another inflammatory marker with a similar efficacy as the sputum eosinophil count in predicting both the short- and long-term responses to corticosteroids.

### Cytokines as Biomarkers of Asthma Severity

Severe asthma is characterized by elevated levels of proinflammatory cytokines and neutrophilic inflammation in the airways. Blood cytokines, biomarkers of systemic inflammation, may be a feature of increased inflammation in severe asthma. One study found that IL-8 and TNF-α levels were higher in severe asthmatics than in

mild-moderate asthmatics or in controls and, in conjunction with augmented circulating neutrophils, suggest the involvement of neutrophil-derived cytokine pattern (Silvestri et al. 2006). Furthermore, in patients with severe asthma, TNF-α level was positively correlated with both eNO and circulating neutrophil counts. Cytokine levels were elevated even though the patients were on high-dose inhaled steroids. This finding might reflect the inability of these drugs to significantly suppress production of this cytokine by airway cellular sources including epithelial cells and inflammatory cells. In patients with severe asthma there may be an imbalance between IL-8 production and the blocking capacity of IL-8 autoantibodies. The findings of this study may be clinically relevant and suggest that drugs that block TNF-α release or activity might represent a new treatment option in severe asthma.

**Exaled NO as a Biomarker of Asthma**

Airway hyperresponsiveness is the main feature of asthma and is defined as an increase in the ease and degree of airway narrowing in response to brochoconstrictor stimuli. Inflammation plays a central role in the pathogenesis of asthma and much of it can be attributed to helper T cell type 2 cytokine activation, the degree of which strongly correlates to disease severity. One of the inflammatory mediators in asthma is NO. The eNO level is elevated in asthma, and can predict asthma exacerbation. It may be clinically more useful to compare exhaled NO values with a subject's previous values than to compare them with a population based normal range.

Cough variant asthma (CVA) and atopic cough both present with bronchodilator-resistant non-productive cough but may be differentiated from and other causes of chronic non-productive cough by measuring exhaled NO. Exhaled NO levels in patients with atopic cough are significantly lower than those in patients with CVA and bronchial asthma (Fujimura et al. 2008). There are no significant difference in the exhaled NO levels between patients with CVA and bronchial asthma.

A UK study findings show that it is feasible to measure bronchial flux NO concentration ($^{J}NO$) and alveolar NO concentration ($C_{alv}$) in 70 % of children, with $C_{alv}$ levels potentially reflecting alveolar inflammation in asthma (Paraskakis et al. 2006). $C_{alv}$ and $^{J}NO$ were measured from the fractional exhaled NO ($FeNO_{50}$) at multiple exhalation flow rates in asthmatic children. Although $FeNO_{50}$ and JNO give essentially the same information, $C_{alv}$ is higher in asthmatic children than in normal children. This study also highlights the relationship between poor control of asthma and $C_{alv}$ (a biomarker of alveolar inflammation) but further work is needed to confirm the relevance of this. Researchers at the University of Pittsburgh, Pennsylvania, have developed a novel nanosensor that can detect a possible asthma attack before it begins. The minute sensor can be fitted into a hand-held device, and when a person blows into the device, it measures the NO content of their breath. Use of this device would provide asthma sufferers with a simple and cost effective way to monitor their asthma inflammation.

An explanation for increased levels of exhaled NO is nonenzymatic generation of NO from nitrite due to airway acidification in asthmatics. Reduced arginine

availability may also contribute to lung injury by promoting formation of cytotoxic radicals such as peroxynitrite. As arginine levels decline, nitric oxide synthase (NOS) itself can begin to generate superoxide in lieu of NO, thereby favoring NO consumption via the generation of peroxynitrite that could induce lung injury. This reduction in bioavailability of NO via formation of species such as peroxynitrite could be further amplified by the rapid loss of SOD activity during the asthmatic response.

Plasma arginase activity declines significantly with treatment and improvement of symptoms. Additional studies are needed to determine whether measurements of plasma arginase activity will provide a useful biomarker for underlying metabolic disorder and efficacy of treatment for this disease. The arginase activity present in serum probably does not accurately reflect whole body arginase activity or that compartmentalized in the lungs, since the arginases are intracellular enzymes. Because arginase is induced in monocytes in response to helper T cell type 2 cytokines, it is speculated that these cells are one likely source of the elevated arginase in serum, consistent with the localization of arginase expression within macrophages in the lungs.

Although exhaled NO is a clinically useful biomarker of eosinophilic airway inflammation in asthma, significant validation and investigation are required before exhaled breath condensate could be utilized for making decisions in clinical practice (Simpson and Wark 2008).

### Endothelin-1 in Exhaled Breath as Biomarker of Asthma

Endothelins are proinflammatory, profibrotic, broncho- and vasoconstrictive peptides, which play an important role in the development of airway inflammation and remodeling in asthma. A study has evaluated the endothelin-1 (ET-1) levels in exhaled breath condensate (EBC) of asthmatics with different degree in asthma severity (Zietkowski et al. 2008). ET-1 concentrations in EBC of all asthmatic patients were significantly higher than in healthy volunteers. ET-1 levels were significantly higher in patients with unstable asthma than in the two groups with stable disease. Thus, measurements of ET-1 in EBC may provide another useful diagnostic tool for detecting and monitoring inflammation in patients with asthma. The release of ET-1 from bronchial epithelium through the influence of many inflammatory cells essential in asthma and interactions with other cytokines, may play an important role in increase of airway inflammation, which is observed after postexercise bronchoconstriction in asthmatic patients.

### IgE as a Biomarker to Guide Dosing of Omalizumab for Asthma

IgE plays a central role in the pathophysiology of asthma. The two essential phases in this pathophysiology are sensitization to allergen and clinical expression of symptoms on reexposure to the sensitizing allergen. Omalizumab (Xolair, Genentech) is a recombinant humanized IgG1 monoclonal anti-IgE antibody that

binds to circulating IgE, regardless of allergen specificity, forming small, biologically inert IgE–anti-IgE complexes without activating the complement cascade. An 89–99 % reduction in free serum IgE (i.e., IgE not bound to omalizumab) occurs soon after the administration of omalizumab, and low levels persist throughout treatment with appropriate doses. A total serum IgE level should be measured in all patients who are being considered for treatment with omalizumab, because the dose of omalizumab is determined on the basis of the IgE level and body weight (Strunk and Bloomberg 2006). The dose is based on the estimated amount of the drug that is required to reduce circulating free IgE levels to less than 10 IU per milliliter.

## *Genotyping in Asthma*

Several clinical trials have highlighted the effects of genotype on response to asthma therapy. Various publications have described the potential of using genotyping as a tool to develop individualized patient treatment regimens for asthma to improve results and limit adverse effects of certain therapies (Lugogo et al. 2007). Increased AHR to bradykinin induced by allergan exposure is due to impaired production of nitric oxide (NO), which is associated with downregulation of eNOS and upregulation of iNOS within the airway epithelium. Polymorphisms of the eNOS gene may be associated with the development of asthma but may not affect the severity of the disease. Recently, a naturally occurring gene mutation has been identified encoding a member of enzymes that appear to be important in the innate immune response and is present in 5–10 % of the normal population. The mutation is a 24 base pair duplication that leads to undetectable mRNA expression in macrophages and a lack of enzyme activity. This role of this mutation has been studied in host immunity to parasitic infections. An assay for the mutation will be useful to gauge an individual's risk for developing asthma and an asthmatic's risk for developing severe asthma. With the rapid progress in the identification of genes involved in various ethnic populations combined with the availability in future of well-targeted drugs, it will be possible to prescribe appropriate medicines for the genetic make-up of an individual.

Collaborative, retrospective, observational health outcomes studies that combine pharmacy, medical claims and genotyping data for participating managed care patients with asthma are focusing on assessing the impact of common genetic variations on clinical outcomes and health care resource utilization for patients using drugs commonly employed for the management of asthma. The results of such studies may provide data indicating whether physicians should consider alternative regimens to improve management of asthma patients with genetic variations. Genotyping of individuals at high risk of developing asthma will enable asthma risk stratification for therapeutic measures to be implemented. In addition, genotyping can be used in clinical trials to assure the comparability of experimental and control populations. Finally, such a genetic asthma test will allow physicians to tailor therapy for asthmatics; aggressive treatment for individuals at risk for severe disease and minimal treatment (avoiding the risk of medication side effects) for those at low risk.

A study that used the clinical data and DNA resources from patients enrolled in the Childhood Asthma Management Program identified a variant in the glucocorticoid-induced transcript 1 gene (GLCCI1), rs37972, associated with a decrease in forced expiratory volume in 1 s (FEV1) in response to treatment with inhaled glucocorticoids (Tantisira et al. 2011). To offer additional reassurance that they had identified a causative SNP, the investigators provided data from isolated cell systems containing the pharmacogenetically identified SNP to show that the presence of these sequence variants was associated with biologic changes that would be consistent with a decreased response to these agents. Approximately 16 % of the population will be homozygous for the genotype responsible for the more limited response to inhaled glucocorticoids. For personalization of treatment of asthma to become a reality, the next step should be to conduct clinical trials in which patients are stratified according to their biologic signatures to determine whether knowledge of this information leads to better clinical outcomes (Drazen 2011).

#### Genetic Polymorphism and Response to $\beta_2$-Adrenergic Agonists

Inhalation of salbutamol, a $\beta_2$-adrenergic agonist that has a bronchodilator effect in asthma, aids the flow of air to the lungs. $\beta_2$-adrenergic receptor gene contains 13 SNP and an analysis of all the possible inter-individual variations has shown that four common differences predict how people respond to salbutamol. This drug works very well in those with one pattern of DNA in a gene that helps to relax muscles in a person's lungs, not at all in those with another, and moderately in the other two groups. However, the issue of whether regular use of an inhaled $\beta_2$-adrenergic agonist worsens airflow and clinical outcomes in asthma is controversial. Retrospective studies have suggested that adverse effects occur in patients with a genetic polymorphism that results in homozygosity for arginine (Arg/Arg), rather than glycine (Gly/Gly), at amino acid residue 16 of the $\beta_2$-adrenergic receptor. A genotype-stratified, randomized, placebo-controlled cross-over trial found that over time the study participants' responses to daily doses of inhaled albuterol differed depending on which form of a specific gene they had inherited (Israel et al. 2004). While a few weeks of regular use of albuterol improved overall asthma control in individuals with one form of the gene, stopping the drug eventually improved asthma control in those with another form of the gene. Genotype at the 16th amino acid residue of the $\beta_2$-adrenergic receptor affected the long-term response to albuterol use. It was recommended that bronchodilator treatments avoiding albuterol may be appropriate for patients with the Arg/Arg genotype.

### *Lebrikizumab for Personalized Treatment of Asthma*

Lebrikizumab (Roche) is an injectable humanized MAb designed to block IL-13, which contributes to key features of asthma. Lebrikizumab improves lung function in adult asthma patients who are unable to control their disease on inhaled corticosteroids.

IL-13 induces bronchial epithelial cells to secrete periostin, a matricellular protein. Increased levels of periostin, a biomarker of asthma, can be measured in the blood. In the MILLY phase II trial, patients with high pretreatment periostin levels had greater improvement in lung function when treated with lebrikizumab, compared to patients with low periostin levels (Corren et al. 2011). The primary endpoint of the trial showed that at week 12, lebrikizumab-treated patients had a 5.5 % greater increase in lung function from the baseline compared to placebo. Lebrikizumab-treated patients in the high-periostin subgroup experienced an 8.2 % relative increase from baseline forced expiratory volume in 1 s (FEV1), compared with placebo. In the low-periostin subgroup, those patients on the drug experienced a 1.6 % relative increase in FEV1, compared with placebo. These results support further investigation of lebrikizumab as a personalized medicine for patients who suffer from moderate to severe uncontrolled asthma periostin enables selection of patients who will benefit most from the drug.

## Personalized Therapy of Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD), which comprises emphysema and chronic bronchitis, is a major public health problem. COPD is defined as low ratio of forced expiratory volume in 1 s (FEV1) to forced vital capacity (FVC) after an inhaled bronchodilator. The grade, or severity, of COPD is based on level of impairment in FEV1 as a percentage of the predicted value, which reflects a decrease in the volume of air forcibly exhaled from the lungs during the beginning of exhalation. COPD affects >16 million Americans and it is the only disease among the top 10 causes of death with a rising mortality rate in the US. It is predicted to be the third largest cause of death by 2020 and has already reached worldwide epidemic proportions. The natural history of this disease is generally characterized by continued decline in lung function, which is highly variable.

### *Biomarkers of COPD*

There has been increasing interest in using pulmonary biomarkers to understand and monitor the inflammation in the respiratory tract of patients with COPD. Bronchial biopsies and bronchoalveolar lavage provide valuable information about inflammatory cells and mediators, but these procedures are invasive, so that repeated measurements are limited. Sputum provides considerable information about the inflammatory process, including mediators and proteinases in COPD, but samples usually represent proximal airways and may not reflect inflammatory processes in distal bronchi. Analysis of exhaled breath is a noninvasive procedure so that repeated measurements are possible, but the variability is high for some assays. There is relatively little information about how any of these biomarkers relate to other clinical outcomes, such as progression of the disease, severity of disease,

clinical subtypes or response to therapy. More information is also needed about the variability in these measurements. In the future pulmonary biomarkers may be useful in predicting disease progression, indicating disease instability and in predicting response to current therapies and novel therapies, many of which are now in development.

Measurements of C-reactive protein (CRP), a biomarker of inflammation, provide incremental prognostic information beyond that achieved by traditional biomarkers in patients with mild to moderate COPD, and may enable more accurate detection of patients at a high risk of mortality (Man et al. 2006). Lung function decline was significantly related to CRP levels, with an average predicted change in FEV1 of −0.93 % in the highest and 0.43 % in the lowest quintile. However, respiratory causes of mortality were not significantly related to CRP levels.

### Alpha1-Antitrypsin Gene Polymorphisms Predisposing to Emphysema

Alpha1-antitrypsin (AAT) is a plasma glycoprotein that inhibits neutrophil elastase, and individuals who inherit altered AAT genes resulting in deficiency of the protein are at high risk for COPD and liver cirrhosis. This deficiency can be detected by serum protein pattern studies. In the past, testing for the deficiency has been done retrospectively in patients with COPD or liver disease, but the introduction of a home-administered finger-stick blood spot test for AAT genotype enables affected families to construct pedigrees to enable them to identify children who are at risk for developing COPD in later life and should avoid exposure to dust and smoke.

### Biomarkers of Lung Failure in COPD

Lung failure, also termed “lung attack”, is the most common organ failure seen in the intensive care unit. Lung attacks, which effect individuals with COPD are among the leading cause of visits to emergency rooms among chronic disease sufferers. Other causes are neuromuscular impairment, pulmonary edema, pneumonia, and vascular diseases such as acute or chronic pulmonary embolism. When a patient is admitted into the hospital with a severe lung failure, it usually takes >3 months to get to 80 % of his or her baseline health. If the patient’s health is poor to start with, the new attack can be devastating or even fatal. A test that could more accurately present a patient's disease could make it easier to predict and treat COPD progression to lung failure. There is need for a test that could be performed in any clinical lab and could be used far more widely than the current lung function tests, which are performed in certain centers by specially trained personnel.

In 2012, Canada’s Prevention of Organ Failure (PROOF) Center of Excellence in Vancouver received funding from Genome British Columbia to develop a biomarker-based test for determining a COPD patient’s risk for having a lung attack. Genes and protein biomarker sets that have been discovered at PROOF Center could have the ability to predict COPD-caused lung attacks and need to be validated.

### Chromagranin A as Biomarker of COPD in Smokers

A study has revealed that serum levels of the neuroendocrine activity biomarker chromagranin A (CgA) are increased in male smokers with impaired lung function, and are associated with both respiratory symptoms and the degree of airway obstruction (Sorhaug et al. 2006). The subgroup of airway epithelial cells belonging to the diffuse neuroendocrine system, termed pulmonary neuroendocrine cells, may represent a putative regulatory function of CgA as a prohormone. They are considered to control growth and development of the fetal lung and regulation of ventilation and circulation, but may also have a role in the pathogenesis of smoking-induced airway disease. The findings indicate that neuroendocrine activation may be important in smoking-related airway inflammation and remodeling, and raise the possibility that CgA could be of predictive value as a biomarker of prognosis in smoking-associated diseases.

## *Gene Expression Studies of Lung Tissue in COPD*

Gene expression analysis using microarrays showed that cigarette smoke induces significant changes in oxidant defense responses in persons who develop COPD (Pierrou et al. 2007). Microarray analysis has demonstrated downregulation of NOTCH pathway-related genes associated with smoking and COPD (Tilley et al. 2009). Whole-genome gene expression is a useful method for studying the molecular changes underlying COPD as well as the heterogeneity among patients with COPD.

Further studies have revealed a 98-gene expression signature of COPD and lung function impairment that reflects disease-associated changes in small airway and lung tissues. Transcriptomic approaches to study the lung tissues in COPD will further improve the knowledge of molecular mechanisms underlying this heterogeneous disease and identify molecular subtypes of disease that have similar clinical manifestations (Steiling et al. 2013).

### Gene Expression Profile in Peripheral Blood of Patients with COPD

Genome-wide expression profiling of peripheral blood samples from subjects with significant airflow obstruction was performed to find non-invasive gene expression biomarkers for COPD (Bhattacharya et al. 2011). Correlation of gene expression with lung function measurements identified a set of 86 genes. A total of 16 biomarkers showed evidence of significant correlation with quantitative traits and differential expression between cases and controls. Further comparison of these peripheral gene expression biomarkers with those previously identified from lung tissue of the same cohort revealed that two genes, RP9 and NAPE-PLD, were decreased in COPD cases compared to controls in both lung tissue and blood. These results contribute to our understanding of gene expression changes in the peripheral blood of patients with COPD and may provide insight into potential mechanisms involved in the disease.

**Increased Expression of PIGF as a Biomarker of COPD**

Decreased expression of vascular endothelial growth factor (VEGF) and its receptor has been implicated in the pathogenesis of COPD. Levels of placenta growth factor (PlGF), another angiogenic factor, are increased in the serum and bronchoalveolar lavage (BAL) fluid of patients with COPD and are inversely correlated with FEV1 (Cheng et al. 2008). Serum levels of PlGF in patients with COPD were more than double those in smokers and nonsmokers without COPD. These findings suggest that bronchial epithelial cells can express PlGF, which may contribute to the pathogenesis of COPD. Both PlGF and VEGF expression levels were increased in cultured bronchial epithelial cells exposed to pro-inflammatory cytokines such as TNFα and IL-8. Although the mechanisms underlying the observed detrimental effects of PlGF remain to be clarified, persistent PlGF expression might have adverse effects on lung parenchyma by down-regulating angiogenesis.

## *Prognosis of COPD*

The BODE index (including body-mass index, airflow obstruction, dyspnea, and exercise capacity) was an important contribution to the prognostic assessment of patients with COPD. However, the BODE index is rarely used in primary care settings where most patient treatment options are managed, because exercise capacity cannot be easily measured in the usual physician's office. The BODE index has been updated to improve its calibration, and a simplified ADO (including age, dyspnea, and airflow obstruction) index was developed for use in primary-care settings (Puhan et al. 2009). Both the updated BODE and ADO indices accurately predicted 3-year mortality and could lend support to the prognostic assessment of patients with COPD in specialized and primary-care settings, e.g. to predict a patient's risk of dying from COPD. Such assessment enhances the targeting of treatments to individual patients.

## *Management of COPD*

Comprehensive management of COPD includes proper assessment, monitoring of disease, reduction of risk factors, the management of stable COPD, as well as the prevention and management of exacerbations. Guidelines from the Global Initiative for Chronic Obstructive Lung Disease address each of these aspects of COPD management in detail and provide evidence-based recommendations for patients and health-care professionals (Gold 2009). Reduction of risk factors emphasizes the importance of smoking cessation and control of environmental indoor and outdoor pollutants. The management of COPD must be individualized.

An improved understanding of the pathomechanism of COPD can be leveraged to develop targeted therapies and ultimately personalize treatment of COPD based on each patient's specific molecular subphenotype. Comparisons between gene expression patterns of various diseases have been used to identify disease-specific pathway dysregulation that can be targeted with pathway-directed medications. This may enable repositioning of established drugs for other diseases for treatment of COPD in addition to discovery of new drugs targeted to pathways affected in COPD.

## Personalized Management of Interstitial Lung Disease

Interstitial lung disease (ILD) is defined as restrictive lung function impairment with radiographic signs of ILD. Idiopathic pulmonary fibrosis (IPF) is the most common and lethal form of the interstitial lung disease. There are currently no effective or approved drugs available to treat it. Diagnosis is by exclusion of other lung diseases and the only definite diagnosis is by lung biopsy but it carries some morbidity and mortality. Lung transplant is the only treatment option but it is available for only a small fraction of IPF patients. Emerging concepts of pathogenesis include the role of cellular senescence, oxidative stress, endoplasmic reticulum stress, microRNAs, and mechanotransduction. Novel variants in TOLLIP and SPPL2C are associated with IPF susceptibility and one novel variant of TOLLIP, rs5743890, is also associated with mortality (Noth et al. 2013). These associations and the reduced expression of TOLLIP in patients with IPF who carry TOLLIP SNPs emphasize the importance of this gene in the disease.

### *Biomarkers of Interstitial Lung Disease*

#### Pulmonary Surfactant Proteins as Biomarkers for Lung Diseases

Pulmonary surfactant, a complex of lipids and proteins, functions to keep alveoli from collapsing at expiration. Surfactant proteins A (SP-A) and D (SP-D) belong to the collectin family and play pivotal roles in the innate immunity of the lung. Pulmonary collectins directly bind with broad specificities to a variety of microorganism and possess antimicrobial effects. These proteins also exhibit both inflammatory and antiinflammatory functions. The collectins enhance phagocytosis of microbes by macrophages through opsonic and/or non-opsonic activities. The proteins stimulate cell surface expression of phagocytic receptors including scavenger receptor A and mannose receptor. Since the expression of SP-A and SP-D is abundant and restricted within the lung, the proteins are now clinically used as biomarkers for lung diseases. The levels of SP-A and SP-D in bronchoalveolar lavage fluids,

amniotic fluids, tracheal aspirates and pleural effusions reflect alterations in alveolar compartments and epithelium, and lung maturity. The determination of SP-A and SP-D in sera is a noninvasive and useful tool for understanding some pathological changes of the lung in the diseases, including pulmonary fibrosis, collagen vascular diseases complicated with interstitial lung disease, pulmona ry alveolar proteinosis, acute respiratory distress syndrome and radiation pneumonitis (Takahashi et al. 2006).

**Serum KL-6 as Biomarker of Interstitial Lung Disease**

KL-6, a mucinous high-molecular weight glycoprotein, is expressed on type II pneumonocytes and is a potential biomarker of ILD. Retrospective, cross-sectional analysis of Caucasian patients with polymyositis (PM) or dermatomyositis (DM) and ILD showed elevated serum levels of KL-6 compared to patients without ILD (Fathi et al. 2012). At a cut-off level of 549 U/ml, the sensitivity and specificity for diagnosis of ILD was 83 % and 100 %, respectively. The level of serum KL-6 may serve as measure of ILD in patients with PM/DM, and is a promising biomarker for use in clinical practice to assess response to treatment.

## *Developing Personalized Therapies for Interstitial Lung Disease*

There is a need for therapeutic approaches that target molecular pathways to modulate aberrant processes and promote tissue homeostasis in the lung. The diversity of biological and clinical phenotypes of IPF requires a personalized medicine approach for diagnosis and treatment of this disorder (Ding et al. 2011). However, the complex tasks of making a definite diagnosis of a specific form of interstitial lung disease and formulating a patient-centered, personalized management plan in an attempt to achieve remission or stabilization of the disease process poses a challenge to clinicians (Meyer 2014). Suggestions that have been made to personalize and improve therapy of IPF are (Herazo-Maya and Kaminski 2012):

- To identify biomarkers specific to IPF to improve the diagnosis and reduce the need for costly and sometimes dangerous interventions, as well as identify patients who may response to specific therapeutic modalities.
- To develop novel antifibrotic agents that can be tested in well-designed, randomized, and “personalized” clinical trials.

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# Chapter 16
# Personalized Management of Genetic Disorders

## Introduction

Classical genetics has blended with molecular biology to produce the revolutionary new field called molecular genetics. A large number of diseases have a genetic component: they are either called genetic disorders (single gene defect) or have a genetic predisposition as a part of multifactorial etiology. Role of genetics in the development of personalized medicine has been discussed in Chap. 1. Molecular diagnostic technologies provide the possibility of preimplantation diagnosis and prevention of birth of affected offspring. Those missed at this stage could be detected in prenatal diagnosis giving the parents an option in decision making for continuation of the pregnancy. Specific treatments for correction of effects of genetic defects are available for some diseases and gene therapy is being developed for single gene disorders. Some of the genetic disorders are described in other chapters dealing with various systems such as the nervous system.

## Molecular Diagnosis of Genetic Disorders

Currently, there are >2,500 genetic tests to detect the risk of disease but the number is much smaller for approved and marketed tests. Several are used in clinical research, many more are in various stages of development, and some are expected to be available in the near future. The term diagnosis should be distinguished from screening, which is integral to all medical evaluations. Conventional genetic screening has severe limitations. Clinical features can be ambiguous and may take years to evolve. The biochemical tests are expensive and often give equivocal results.

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Thus, these traditional approaches had severe limitations for the identification of carriers and prenatal diagnosis. The tools of molecular genetics are beginning to permit genotypic screening. The most practical outcome of the use of recombinant DNA technology in medical genetics has been improved diagnosis and prediction of inherited diseases, e.g. ALS, Huntington disease, cystic fibrosis, breast cancer and hemophilia. Other applications are in disease management and pharmacogenomics.

Molecular diagnostic methods are described in a special report on this topic (Jain 2015d). A few that are used for diagnosis of genetic disorders will be mentioned in this Chapter. Advantages of molecular diagnosis in genetic disorders are:

- The presence or absence of a mutation in an affected person or a carrier can be determined without any ambiguity.
- A distinction can be drawn between disorders with similar phenotypes.
- Diagnosis can be made in advance of clinical manifestations.
- The high specificity of molecular diagnostics permits screening of large populations to identify carriers.
- In this application, molecular genetic tests are not usually directed at a particular anatomical lesion, as DNA obtained from anybody site is equally valid.
- Tests can be used as the basis for gene therapy. If a normal gene has been cloned for use as a probe for disease-causing mutations, the same normal sequence can be used to replace the mutated sequence in the patient. This type of gene repair has been tried experimentally in cystic fibrosis by using in situ PCR.

The main limitations of molecular diagnostics for genetic disorders are as follows:

- Because the genetic changes that underlie inherited disorders are so heterogeneous, the mutations can be so diverse that no two persons will demonstrate the same change. This variability hinders the construction of a molecular diagnostic test that is applicable to all patients with a certain disease. For example, two distinct genes have been implicated in tuberous sclerosis in different families.
- A normal counterpart may mask the gene deletion. Because non-sex-linked genes come in pairs, the normal gene can potentially hide the loss of part or all of the other copy. In conventional gene testing, PCR amplifies the intact gene inherited from one parent but the normal copy hides the mutated gene from the other parent.

## *Molecular Diagnostic Technologies*

### Cytogenetics

In the past decade, clinical cytogenetics has undergone remarkable advancement as molecular biology techniques have been applied to conventional chromosome analysis. The limitations of conventional banding analysis in the accurate diagnosis and

interpretation of certain chromosome abnormalities have largely been overcome by these new technologies, which include fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and multicolor FISH (M-FISH, SKY, and Rx-FISH). Clinical applications include diagnosis of microdeletion and microduplication syndromes, detection of subtelomeric rearrangements in idiopathic mental retardation, identification of marker and derivative chromosomes, prenatal diagnosis of trisomy syndromes, and gene rearrangements and gene amplification in tumors. Molecular cytogenetic methods have expanded the possibilities for precise genetic diagnoses, which are extremely important for clinical management of patients and appropriate counseling of their families. Cytogenetics is dealt with in more detail in a special report on this topic (Jain 2015b).

### FISH with Probes to the Telomeres

When clinicians see a child with developmental delay or mental retardation and any kind of congenital anomaly, the first thing they consider is chromosomes. Basic chromosome analysis does not always catch genetic disorders. For example, the 1p36 deletion syndrome in which a deletion occurs on the telomere or tip of chromosome 1, is missed because genetic loss occurs on the most telomeric or distal band of chromosome 1. But the syndrome has clinically recognizable aspects – facial characteristics, seizures, mental retardation, hearing loss and slow developmental growth. These signs have been correlated with 1p21-22 deletion by use of a refined FISH technique with probes to the telomeres. Nearly half of the cases now diagnosed by use of refined FISH were previously missed by chromosome analysis that was reported normal. Early diagnosis is important as it would lead to early intervention and therapies, and help the patient and family deal with a particular disorder.

### Single Copy FISH Probes

Current commercially produced DNA probes, while important and useful, are limited to primarily examining large sections of DNA in identifying relatively common genetic disorders. Single copy FISH (scFISH) probes offer specificity in hybridizing genetic chromosomes not heretofore available for identifying elusive strains of inherited genetic diseases. Enzo Biochem Inc has acquired a license to the technology from the Children's Mercy Hospital & Clinics (Kansas City, MO) along with rights to ~50 DNA probes developed at the University of Missouri, which have been shown to identify a number of genetic diseases. scFISH probes were designed by computational sequence analysis of ~100-kb genomic sequences, produced by long PCR, then purified, labeled, and hybridized individually or in combination to human chromosomes. Preannealing or blocking with unlabeled, repetitive DNA is unnecessary, as scFISH probes lack repetitive DNA sequences. The hybridization results are analogous to conventional FISH, except that shorter probes can be readily visualized. Combinations of probes from the same region gave single hybridization

signals on metaphase chromosomes. ScFISH probes are produced directly from genomic DNA, and thus more quickly than by recombinant DNA techniques. Single-copy probes have been developed for three chromosomal regions: the CDC2L1 (chromosome 1p36), MAGEL2 (chromosome 15q11.2), and HIRA (chromosome 22q11.2) genes. Abnormalities seen on metaphase chromosomes could be characterized with scFISH probes at a resolution greater than previously possible.

Visualization of human chromosomes is a routine laboratory procedure but commercially available FISH probes, covering 100–300 kilobases, or 100,000–300,000 DNA bases, are too large for their targeted DNA. Single copy probes, by contrast, are much smaller and more densely represented on a chromosome. They can, therefore, detect smaller lesions in addition to being able to probe rare conditions, whereas current clinically available probes principally detect relatively common abnormalities. Single copy probes thus can enable more precise treatments for individuals, even differentiating between two patients suffering from what may otherwise appear to be the same disease. In addition, because the single copy probes are very small and derive directly from the genome sequences, they can precisely localize chromosomal breakpoints based on which chromosome harbors the hybridized signal.

### Comparative Genomic Hybridization

Comparative genomic hybridization (CGH) is a modified in situ hybridization technique, which allows detection and mapping of DNA sequence copy differences between two genomes in a single experiment. In CGH analysis, two differentially labeled genomic DNA (study and reference) are co-hybridized to normal metaphase spreads. Chromosomal locations of copy number changes in the DNA segments of the study genome are revealed by a variable fluorescence intensity ratio along each target chromosome. Since its development, CGH has been applied mostly as a research tool in the field of cancer cytogenetics to identify genetic changes in many previously unknown regions. CGH may also have a role in clinical cytogenetics for detection and identification of unbalanced chromosomal abnormalities. It is now possible to confirm euploidy at the time of implantation in a woman undergoing in vitro fertilization by using CGH with the pregnancy to ensure that the offspring will be free from congenital disorders.

### Representational Oligonucleotide Microarray Analysis

Representational oligonucleotide microarray analysis (ROMA) was developed by arraying oligonucleotide probes designed from the human genome sequence, and hybridizing with "representations" from cancer and normal cells to detect regions of the genome with altered "copy number". ROMA is used to describe copy number changes in patients with chromosomal abnormalities and can define cytogenetic

aberrations with extraordinary precision. It will assist in the discovery of genes and markers important in cancer, and the discovery of loci that may be important in inherited predispositions to disease. Together with the information from the human genome sequence and proteomics, ROMA will provide the ability to define rearrangements with ultra-high resolution will improve the ability to provide accurate prognosis both prenatally and postnatally to parents of offspring with chromosomal aberrations.

### Diagnosis of Genomic Rearrangements by Multiplex PCR

Germline and somatic genomic rearrangement play a relevant role in the pathogenesis of genetic disorders, and their identification is a fundamental task in molecular diagnosis. However, screening for structural genomic abnormalities is often not included in routine mutational analyses and consequently the proportion of rearrangements playing a pathogenic role in several genetic disorders is likely to be underestimated. A wide range of molecular techniques for the detection of large genomic rearrangements has been developed: some have the power to screen the whole genome, others are designed to analyze one or few loci that are known to be involved in a specific disease; some may detect balanced rearrangements, while others only unbalanced rearrangements; some are suitable for detection of germline abnormalities, yet others also detect somatic abnormalities. Multiplex PCR-based protocols are currently employed in routine detection of extended germline genomic deletions or duplications (De Lellis et al. 2008).

### Mutation Detection Technologies

Procedures for mutation detection can be separated into two distinct groups. The first group consists of methods to scan sequences for all mutations including known and unknown disease causing alleles. The second consists of single nucleotide polymorphism (SNP) technologies (see Chap. 2), which efficiently detect known and common disease causing alleles and are described in the next section. Some of the technologies in two groups overlap.

Traditionally SNP technologies had the advantage of being able to detect known mutations inexpensively, and with high reproducibility. The problem with using these technologies in general is the inability to detect rare disease causing mutations, which on the whole, can account for a significant number of diseased individuals. An example is the cystic fibrosis (CF) testing services based on this technology, which fails to identify a large number of disease causing alleles, especially in rare CF occurring ethnicities.

Traditionally the mutation scanning and sequencing methods have been expensive and/or not reproducible. Mutation detection technologies are shown in Table 16.1 and some of these are described in Chap. 2.

**Table 16.1** Mutation detection technologies

| |
|---|
| **Polymerase chain reaction (PCR)-based methods** |
| Amplification of refractory mutation system (ARMS) |
| Cleavase fragment length polymorphism (CFLP) |
| Digital genetic analysis (DGA) |
| Direct dideoxy sequencing (DDS) |
| Fluorescence-based directed termination PCR |
| Heteroduplex analysis (HA) and its other versions |
| Denaturing gradient gel electrophoresis (DGGE) |
| WAVE system |
| Multiplex allele-specific diagnostic assay (MASDA) |
| Non-isotopic RNase cleavage assay (NIRCA) |
| Primer extension dependent isothermal amplification technology |
| Restriction fragment length polymorphism (RFLP) |
| Single-strand conformational polymorphism (SSCP) |
| TaqMan real-time PCR |
| **Non-PCR methods** |
| Arrayed primer extension (APEX) |
| BEAMing (beads, emulsion, amplification, and magnetics) |
| Conversion analysis for mutation detection |
| Enzyme mutation detection (EMD) |
| Peptide nucleic acid (PNA) technology |
| Specific anchor nucleotide incorporation |
| **Biochip technologies** |
| **Haplotype specific extraction (HSE)** |



## Biomarkers for Genetic Disorders

There are a large number of genetic disorders where biomarkers are used along with molecular diagnostics for screening and diagnosis. These are described in more details in the report on biomarkers (Jain 2015a). A few examples will be described briefly.

### *Biomarkers for Down's Syndrome*

Down's syndrome is a genetic disorder caused by the inheritance of three copies of the 21st chromosome. It is the most common congenital disorder with impairment of mental function; a large percentage of these individuals develop Alzheimer's disease in the fifth decade of life. There is some controversy about the best approach to screening for Down's syndrome. The competing claims of advocates of different screening approaches have made it difficult for health planners, clinicians, or pregnant women to reach a balanced decision about what should be offered, or chosen.

Serum tests used to screen for Down's syndrome include β-human chorionic gonadotrophin (hCG), alpha-fetoprotein (AFP), unconjugated estriol (uE3), serum pregnancy associated plasma protein-A (PAPP-A), and dimeric inhibin A. ADAM12, a novel serum marker with biological properties similar to PAPP-A.

The Serum, Urine and Ultrasound Screening Study (SURUSS) advanced our knowledge of the efficacy and safety of antenatal screening for fetal Down's syndrome and placed choices on a firmer platform of evidence. The best performer was the integrated test, comprising ultrasound measurement of fetal nuchal translucency and assay of PAPP-A at 10 weeks, combined with quadruple tests of serum α-fetoprotein, unconjugated estriol, hCG, and inhibin-A during the second trimester (after 14 weeks). This two step package had a false positive rate of only 0.9 %. The best first trimester screening package was a combination of nuchal translucency scan, serum free β-HCG, and pregnancy associated plasma protein A, which had a false positive rate of 4.3 %. Second trimester quadruple testing alone had a false positive rate of 6.2 %.

Quadruple Marker Prenatal Screening Test (Laboratory Corporation of America) is a blood screening test done in the second trimester of pregnancy (between 15 and 20 weeks) to help detect an increased risk for Down's syndrome, trisomy 18, and neural tube defects or abdominal wall defects. Occasionally, the test may also detect a risk for other chromosome abnormalities. This test measures the concentrations of four biochemical substances produced by the fetus and placenta, AFP, hCG, uE3, and dimeric inhibin A. The test values, together with maternal age, are then entered into a mathematical formula to determine the risk for the various abnormalities. By adding a fourth marker to the prenatal screening test, the detection rate for an elevated risk of Down's syndrome can be increased from 60 % to 75 %.

## *Biomarkers for Muscular Dystrophy*

Duchenne and Becker muscular dystrophy (DMD and BMD) share clinical symptoms like muscle weakness and wasting but differ in clinical presentation and severity. Immunohistochemistry using antibodies to dystrophin is the pathological basis for the diagnosis of DMD and BMD. While the sarcolemma of DMD muscle is negative, BMD muscle generally shows variable labeling because of the translation of a partially functional dystrophin that is localized to the sarcolemma. In some cases this differentiation is not possible. In such instances immunolabeling with antibodies to the neuronal form of nitric oxide synthase (nNOS) can be useful in suspecting a dystrophinopathy with a mutation in the 'hot-spot' rod domain and help to direct molecular analysis. nNOS localizes to the sarcolemma of mature muscle fibers via several components of the dystrophin-associated protein complex including dystrophin but sarcolemmal nNOS is lost when dystrophin levels are very low or absent because of deletions in critical regions of the rod domain.

Gene expression profiling of hind limb muscles of mouse models of muscular dystrophies can clearly discriminate between severely affected and mildly or nonaffected animals. Dystrophin-deficient and sarcoglycan-deficient profiles are remarkably similar, sharing inflammatory and structural remodeling processes. These processes were also ongoing in dysferlin-deficient animals, although at lower levels, in agreement with the later age of onset of this muscular dystrophy. The inflammatory proteins Spp1 and S100a9 were up-regulated in all models. This study has identified biomarker genes for which expression correlates with the severity of the disease. This comparative study is an important step toward the development of an expression profiling-based diagnostic approach for muscular dystrophies in humans.

## *Biomarkers of Phenylketonuria*

Phenylketonuria (PKU) is a genetic disease affecting 1:10,000–14,000 live births. In this condition, phenylalanine hydroxylase (PAH) deficiency is inherited as an autosomal recessive trait and the associated hyperphenylalaninemia phenotype is highly variable. Neurological abnormalities in phenylketonuria include tremor, clumsiness, epilepsy, spastic paraparesis and intellectual impairment. Screening for PKU was introduced in the UK >30 years ago and has proved successful in preventing severe mental retardation. Genotype-based prediction of the biochemical phenotype is now feasible in the majority of newborns with hyperphenylalaninemia, which may be useful for refining diagnosis and anticipating dietary requirements. Methods currently used to screen for PKU include spectrophotometry, fluorometry, immunoassay, and tandem mass spectrometry with electrospray ionization. Developments in tandem mass spectrometry have made it technically possible to screen for several inborn errors of metabolism in a single analytical step. NeoLynx Screening Application-Manager (Waters Corporation) is indicated for the quantitative measurement of phenylalanine and tyrosine in neonatal blood samples by tandem mass spectrometry – exclusively with Quattro micro/Quattro LC mass spectrometers. Additionally, measurements of tyrosine can be used as an adjunct to the measurement of phenylalanine in reducing the number of false-positive results with NeoLynx Screening Application-Manager.

## *Genetic Biomarkers for Psoriasis*

Psoriasis is a common, immune-mediated genetic disorder of the skin and is associated with arthritis in ~30 % of cases. PSORS2 (psoriasis susceptibility locus 2) has been localized to chromosomal region 17q25.3-qter after a genome-wide linkage scan in a family of European ancestry with multiple cases of psoriasis and psoriatic arthritis. In caspase recruitment domain family, member 14 (CARD14), the same authors identified unique gain-of-function mutations that segregated with psoriasis

by using genomic capture and DNA sequencing (Jordan et al. 2012). The mutations altered splicing between CARD14 exons 3 and 4. CARD14 activates nuclear factor kappa B (NF-kB), and compared with wild-type CARD14, the p.Gly117Ser and p.Glu138Ala substitutions were shown to lead to enhanced NF-kB activation and upregulation of a subset of psoriasis-associated genes in keratinocytes. These genes included chemokine (C-C motif) ligand 20 (CCL20) and IL-8. CARD14 is localized mainly in the basal and suprabasal layers of healthy skin epidermis, whereas in lesional psoriatic skin, it is reduced in the basal layer and more diffusely upregulated in the suprabasal layers of the epidermis. The authors propose that, after a triggering event that can include epidermal injury, rare gain-of-function mutations in CARD14 initiate a process that includes inflammatory cell recruitment by keratinocytes. This perpetuates a vicious cycle of epidermal inflammation and regeneration, a cycle which is the hallmark of psoriasis. The identification of the gene and its associated pathways/proteins open an avenue of therapeutic targets for drug development in psoriasis with the hope that a more specific and effective therapy can be developed.

## *Biomarkers of Lysosomal Storage Disorders*

Although several therapies are available or in development for lysosomal storage disorders (LSDs), assessment of therapeutic efficacy is limited by the lack of biomarkers to assess disease progression and severity. This is particularly true for rare diseases such as LSDs, since natural history data from human populations are often lacking. Gene expression analysis in the acid sphingomyelinase-deficient mouse model (ASMKO) of Types A and B Niemann-Pick disease (NPD) has been used to identify novel serum biomarkers (Dhami et al. 2006). Microarray and real-time PCR analyses were used to compare mRNA expression in ASMKO and normal mice in two important sites of pathology, lung and brain, and from these data identified and validated several potential biomarkers. The cytokine MIP-1α was markedly elevated in ASMKO mouse serum, and following enzyme replacement therapy (ERT) it was reduced to normal levels. Total iron levels were similarly elevated in ASMKO mice, reflective of the elevated ferritin light chain transcript, and decreased to normal after ERT. Serum growth hormone levels were also elevated in ASMKO mice and were reduced to normal after brain-directed gene therapy, but not ERT. These studies illustrate the value of gene expression analysis for the identification of biomarkers, and provide new insight into the pathobiology of NPD.

The mucopolysaccharidoses (MPS) is another group of LSDs presenting with broad multi-system disease and a continuous range of phenotypes. Currently, there are no objective biomarkers of MPS disease that clearly reflect disease severity or therapeutic responsiveness. Formation of the heparin cofactor II-thrombin (HCII-T) complex, a well-known serine protease inhibitor (serpin)-serine protease complex, has been identified as an informative biomarker for MPS I by using proteomic studies in the murine MPS I model. HCII-T complex was also elevated in plasma from MPS I patients. The degree of HCII-T complex formation appears to correlate with

disease severity and is responsive to therapy. In addition to its role as a biomarker, the discovery of increased serpin-serine protease complex formation provides a valuable insight into possible pathophysiological mechanisms of MPS.

Gaucher's disease is the most common LSD. Gene defect leads to deficiency or decreased activity of glucocerebrosidase followed by the accumulation of glucosylceramide. Frequent manifestations are hepatosplenomegaly, anemia, skeletal and hematological abnormalities. Recently used enzyme replacement therapy (imiglucerase) seems to eliminate the need for bone marrow transplantation and has favorable effects on symptoms and outcome. Development of gene therapy (reintroduction of missing DNA sequence) offers the possibility of cure of the disease. The biochemical markers secreted by Gaucher's cells are numerous, but none of those identified to date has offered all the expected qualities of a biomarker. Chitotriosidase and chemokine CCL18 are the most useful biomarkers to monitor enzyme replacement therapy. The identification of new biomarkers in the near future should enable a clearer understanding of the pathophysiology of this complex disease, which involves numerous cell processes.

Fucosidosis, another LSD, is an autosomal recessive disorder resulting from a deficiency of α-L-fucosidase, encoded by the FUCA1 gene, which leads to failure in the catabolism of glycoproteins and glycosphingolipids resulting in the accumulation of a range of fuco-oligosaccharides and sphingolipids in all tissues including brain and liver. Severely affected patients present within the first year of life with mental retardation, growth retardation, and abnormalities in various other organs. Most of the diagnostic procedures are either invasive or impractical. PCR can be useful only once the mutation is known. The most practical test is detection by mass spectrometry of the elevated oligosaccharides as a biomarker in urine.

Prenatal diagnosis is available for many LSDs using chorionic villus samples or amniocytes. Such diagnoses can be problematical if sample transport and culture are required prior to analysis. It is possible to identify useful biomarkers for the diagnosis of LSDs from amniotic fluid. Each disorder produces a unique signature metabolic profile of protein, oligosaccharide, and glycolipid biomarkers. Some metabolite elevations directly related to the disorder whilst others appeared unrelated to the primary defect. Many LSDs are clearly distinguishable from control populations by the second trimester and in one case in the first trimester. Samples from GM1 gangliosidosis and mucopolysaccharidosis type VII display a correlation between gestational age and amount of stored metabolite. These results provide proof of principal for the use of biomarkers contained in amniotic fluid as clinical tests for some of the more frequent LSDs.

## Sequencing in Genetic Disorders

An introduction to DNA sequencing and its relation to personalized medicine were discussed in Chap. 2. Details of sequencing technologies are described in a special report on this subject (Jain 2015c). Applications of sequencing in genetic disorders are given in this chapter.

Mendelian diseases are considered to be rare, yet genetic disorders are estimated to occur at a rate of 40–82 per 1,000 live births. Epidemiologic studies show that if all congenital anomalies are considered as part of the genetic load, then ~8 % of persons are identified as having a genetic disorder before reaching adulthood. Most of these cases remain undiagnosed. Genomic sequencing with the use of massively parallel NGS is an effective alternative to locus-specific and gene-panel tests in a research setting for establishing a genetic basis of disease. Initial applications of NGS to clinical diagnosis posed many challenges. Technical, bioinformatic, interpretive, and validation pipelines have been developed for whole-exome sequencing (WES) in a certified clinical laboratory to identify sequence variants underlying disease phenotypes in patients (Yang et al. 2013). Application of WES to the diagnoses of 250 unselected consecutive patients resulted in a molecular diagnostic yield of 25 %, which is higher than the positive rates of other genetic tests, such as karyotype analysis (5–15 %), chromosomal microarray analysis (15–20 %), and Sanger sequencing for single genes (3–15 %). Among the 500 additional clinical exomes completed during the review process, the authors obtained a similar diagnostic yield (26 %). Results of this study support the use of WES as a diagnostic test for patients with nonspecific or unusual disease presentations of possible genetic cause and for patients with clinical diagnoses of heterogeneous genetic conditions. Cost-effectiveness, accuracy, yield, and integration of genome-based diagnosis in medical care must be addressed in future studies and will require prospective study designs. Although this seems logical, a prospective study design involving a million variants per person, long-term follow-up periods for proving effectiveness, and sufficient power to test whether knowledge of any given variant has an effect on clinical outcome would be a challenge with traditional randomized, case control designs. Appropriate study designs that can distinguish pathogenic from benign variants and test the effect of genetic knowledge on clinical outcomes require serious consideration (Jacob 2013).

## *DNA Sequencing for Prenatal Disorders*

In high-risk pregnant women, noninvasive prenatal testing with the use of massively parallel sequencing of maternal plasma cell-free DNA (cfDNA) accurately detects fetal autosomal aneuploidy. In a blinded multicenter study in the US, blood samples from women with singleton pregnancies who were undergoing standard aneuploidy screening (serum biochemical assays with or without nuchal translucency measurement) were collected and massively parallel sequencing was performed to determine the chromosome dosage for each sample (Bianchi et al. 2014). The results showed that prenatal testing with use of cfDNA had significantly lower false positive rates and higher positive predictive values for detection of trisomies 21 and 18 than standard screening with ultrasound and biochemical blood tests. Positive results from standard screening methods, such as ultrasound and testing the mother's blood for proteins associated with fetal deformities, accurately point to Down syndrome in only about 4 % of cases. And if one of those screens indicates a problem, invasive methods, such as amniocentesis, must be performed to substantiate the results.

The study's authors found that cfDNA testing >1,900 pregnant women, however, correctly flagged trisomy 21 > 40 % of the time. If cfDNA testing were to be used as a primary screen, it could result in a 90 % reduction of invasive procedure. A negative result on cfDNA screening obviates the need for invasive testing and thus the discomfort and risk to the pregnancy incurred by such testing (Greene and Phimister 2014). Currently, cfDNA testing is more commonly used in high-risk pregnancies; the new technology is not typically used in all pregnancies.

Verinata Inc's verifi® prenatal test, launched in 2012 through a partnership with Laboratory Corporation of America, quantifies fetal cfDNA fragments. A sequencing-based approach is used to identify chromosomal aneuploidies across the entire genome. Using optimized algorithms, it analyzes DNA sequencing data to identify fetal chromosomal abnormalities: (1) trisomy 21 with 97.2 % sensitivity and 100 % specificity; (2) trisomy 18 (Edwards syndrome) with 100 % sensitivity and specificity; and (3) trisomy 13 (Patau syndrome) with 78.6 % sensitivity and 100 % specificity.

### Sequencing Genomes of the Newborn to Screen for Genetic Disorders

More than 6,000 babies are born every year in the UK with serious developmental disorders, but currently, only a small number of them can be diagnosed before they begin to present patterns of symptoms. In 2011, a project in the UK led by the Wellcome Trust Sanger Institute and the National Health Service (NHS) started work on a plan to analyze the genomes of up to 12,000 children with physical and mental developmental problems and multiple birth malformations in the hopes of developing new tools to diagnose these disorders. The Deciphering Development Disorders (DDD) program will use the resources of all 23 of the NHS Clinical Genetics Services across the UK to collect comprehensive genomic data and to develop clinical tools for diagnosing the genetic causes for these developmental problems. Funded under the Health Innovation Challenge Fund, a partnership between the Department of Health and the Wellcome Trust, the 5-year effort will incorporate the genomic data on these children with information about their physical and mental characteristics. By linking together the expertise in genomics at the Wellcome Trust Sanger Institute with the unique network of Clinical Genetics Services offered by the NHS, these families can directly benefit from the rapid growth in the understanding of human genome. The research will use next-generation sequencing tools to seek out CNVs, exon deletions, and single changes in base pairs that may cause developmental disorders. The DDD project also will lead to an expansion of the DECIPHER (DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources) database, which was started in 2004 to support clinical interpretation of genetic variations and provides the information from clinical centers around the world. The dataset will enable researchers studying child development to link genetic variants to phenotypes and to identify potential molecular targets for treatments, as well as for diagnostics tools. The DDD project will also carry out research with patients and health professionals to identify and analyze the ethical issues likely to arise in the use of these tools and to work

towards the development of appropriate models of good practice in the care of patients and their families.

Monogenic diseases are frequent causes of neonatal morbidity and mortality, and disease presentations are often undifferentiated at birth. Faulty genes for more than ~3,500 monogenic diseases out of the ~7,500 known genetic diseases have been characterized, but clinical testing is available for only some of them and many feature clinical and genetic heterogeneity. Treatment is available for only ~500 of these. Hence, an immense unmet need exists for improved molecular diagnosis in infants. Approximately 1 in 20 babies in newborn intensive care units has a genetic disease, which is difficult to diagnose. Because disease progression is extremely rapid, albeit heterogeneous, in newborns, molecular diagnoses must occur quickly to be relevant for clinical decision-making. A 50-h differential diagnosis of genetic disorders by WGS has been described that features automated bioinformatic analysis and is intended to be a prototype for use in neonatal intensive care units (Saunders et al. 2012). Retrospective 50-h WGS identified known molecular diagnoses in two children. Prospective WGS disclosed potential molecular diagnosis of a severe GJB2-related skin disease in one neonate; BRAT1-related lethal neonatal rigidity and multifocal seizure syndrome in another infant; identified BCL9L as a novel, recessive visceral heterotaxy gene (HTX6) in a pedigree; and ruled out known candidate genes in one infant. Sequencing of parents or affected siblings expedited the identification of disease genes in prospective cases. With the new method, a computer program searches for genes based on the baby's symptoms. Because it focuses only on genes that cause diseases in newborns, it avoids the ethical problem of findings that are unrelated to the problem at hand. Thus, rapid WGS can potentially broaden and foreshorten differential diagnosis, resulting in fewer empirical treatments and faster progression to genetic and prognostic counseling. The method is expensive, though, costing about $13,500. It is not yet covered by insurance. Illumina has a new sequencer that could sequence DNA in 25 h and it will further reduce the time for sequencing.

In the next 5 years, advances in sequencing technology will enable affordable sequencing of the genomes of the four million infants born each year in the US alone, which sequences will serve as a universal and complete genetic test to be used throughout individuals' lives to improve their development and help them lead healthier lives. As such, a newborn's sequence should ideally be obtained as early as possible to reduce potential health and developmental risks. However, personal genomic information will be useful only to the extent that the associations between the genetic sequence and diagnosis or prognosis of a disease can be accurately made in large numbers of people. Most of these association studies have yet to be carried out, but one can foresee that improved diagnostic and prognostic methods would lead to superior health economics and patient outcomes, despite the likelihood of finding a "healthy" genome in the majority of newborns. Alternatively, ignoring the genetic indicators of potential disease risk would almost certainly result in much higher costs, not only for patients but also for governments or insurance companies as compared to the cost of sequencing and analyzing a genome. With a positive healthcare economics rationale, governments or insurance companies will choose to pay for genomic sequencing as health-screening.

### *Study of Rare Variants in Pinpointing Disease-Causing Genes*

Genome-wide association studies (GWAS) use gene chips in automated systems that analyze about 500,000 to one million sites where SNPs tend to occur. In using these SNP chips over the past decade in comparing DNA samples between healthy subjects and patients, scientists have identified thousands of SNPs that associate with common complex diseases. However, SNPs investigated by the gene chips do not themselves cause a disease, but instead serve as a marker linked to the actual causal mutations that may reside in a nearby region. After a GWAS finds SNPs linked to a disease, researchers then perform a "fine-mapping" study by additional genotyping, i.e. sequencing of the gene regions near the SNP signal, to uncover an altered gene that harbors a mutation responsible for the disease.

GWAS have been successful in identifying disease susceptibility loci, but pinpointing of the causal variants in subsequent fine-mapping studies remains a challenge. A conventional fine-mapping effort starts by sequencing dozens of randomly selected samples at susceptibility loci to discover candidate variants, which are then placed on custom arrays and algorithms are used to find the causal variants. A new study challenges the prevailing view that common diseases are usually caused by common gene variants (mutations) but the culprits may be numerous rare variants, located in DNA sequences farther away from the original "hot spots" than scientists have been accustomed to look (Wang et al. 2010). The authors propose that one or several rare or low-frequency causal variants can hitchhike the same common tag SNP so that they may not be easily unveiled by conventional efforts. They demonstrated that the true effect size and proportion of variance is explained by a collection of rare causal variants, which can be underestimated by a common tag SNP, thereby accounting for some of the "missing heritability" in GWAS. Sequencing DNA in subset of patients most likely to carry causative mutations leads to identification of more actual mutations. This refined technique may identify individuals more likely to have mutations in causal genes. By applying their methods to real DNA samples from patients with genetic hearing loss, the researchers' approach helped them to select from GWAS data a subset of cases for sequencing analysis that were most likely to carry causative mutations. Sequencing the DNA in this subset, the study team found that the majority of those patients carried an actual mutation known to cause hearing loss. This approach will facilitate personalized medicine, in which treatment will be tailored to an individual's genetic profile. Identifying causal variants in disease genes provides an opportunity to develop drugs to rectify the biological consequences of these mutated genes.

GWAS have identified multiple loci associated with plasma lipid concentrations. Common variants at these loci together explain <10 % of variation in each lipid trait. Rare variants with large individual effects may also contribute to the heritability of lipid traits. A study has shown an accumulation of rare variants, or a mutation skew, in GWAS-identified genes in individuals with hypertriglyceridemia (Johansen et al. 2010). Through GWAS, the authors identified common variants in APOA5, GCKR, LPL and APOB associated with hypertriglyceridemia. Resequencing of these genes revealed a significant burden of rare missense or nonsense variants in

individuals with hypertriglyceridemia, compared to variants in controls, corresponding to a carrier frequency of 28.1 % of affected individuals and 15.3 % of controls. Consideration of rare variants in these genes incrementally increased the proportion of genetic variation contributing to hypertriglyceridemia.

#### Discovery of the Gene for Miller Syndrome

WES has been applied successfully to discover the gene for a rare mendelian disorder of unknown cause, Miller syndrome, which is characterized by an under-sized jaw, droopy eyes, cleft lip or palate, incomplete or unusual limb development (Ng et al. 2009). The study identified a single candidate gene, DHODH, which encodes a key enzyme in the pyrimidine de novo biosynthesis pathway. Exome sequencing of a small number of unrelated affected individuals is a powerful, efficient strategy for identifying the genes underlying rare mendelian disorders and will likely transform the genetic analysis of monogenic traits. The unique value of complete genome sequencing in families was demonstrated by results of another study to identify mutations underlying Miller syndrome and ciliary dyskinesia, an inherited lung disorder in two affected siblings and their parents (Roach et al. 2010). Along with the disease-related mutations that were detected, the study was also able to use the family's genome sequences to begin exploring the DNA mutation rate from one generation to the next. It is now possible to see all the genetic variations, including rare ones, and to construct the inheritance of every piece of the chromosomes, which is critical for understanding the traits that are important in health as well as disease. Thus the analysis of a family's genome can aid in the diagnosis and treatment of individual family members. It is possible that family's genome sequence may become a part of an individual's medical records in the future.

## Personalized Cell and Gene Therapies of Genetic Disorders

Personalized biological therapies were described in Chap. 9. This chapter will include brief description of applications of personalized cell and gene therapies in some genetic disorders.

### *Personalized Stem Cell Transplant for Sickle Cell Anemia*

Sickle cell anemia (SCA) is an inherited disorder of β-globin, resulting in red blood cell rigidity, anemia, painful crises, organ infarctions, and reduced life expectancy. There are anecdotal reports of successful use of UCB stem cells transplant therapy of children suffering from SCD. Although myeloablative allogeneic HSC transplantation is curative in children with SCD, the procedure is unduly toxic in adults. Graft rejection and GVHD are additional barriers to its success.

Allogeneic blood or marrow transplantation (BMT) can cure SCA but is associated with an 8–10 % mortality rate, primarily from complications of marrow-ablative conditioning. SCD patients who happen to develop mixed chimerism after their own bone marrow has been partially ablated, remain symptom free and have much lower complication rate.

LentiGlobin® BB305 (Bluebird Bio) is obtained by inserting a fully functional human βA-T87Q-globin gene into the patient's own CD34+ HSCs by lentiviral vector encoding this gene. An open label, multi-site, single-dose, phase I study in adults with severe SCD is evaluating the safety and efficacy of the LentiGlobin BB305 (NCT02140554).

## *Hurler Syndrome*

Hurler's syndrome is the most severe form of mucopolysaccharidosis type I (MPS IH). Children with this rare metabolic disease usually die by the age of six because they are missing an important enzyme, alpha-L-iduronidase, which leads to progressive damage in the brain, heart, bones, cartilage, liver and corneas. Patients with a milder form of the disease, with no brain involvement, can receive enzyme replacement therapy alone. However, because enzymes do not cross the blood-brain barrier, they cannot repair the brain damage that occurs in more severe forms of the disease.

Hematopoietic cell transplantation (HCT) is a life-saving measure in MPS IH, but a suitable hematopoietic donor is difficult to find. Because there is no known benefit of immune reaction between the host and the donor cells in MPS IH, gene-corrected autologous stem cells may be the ideal graft for HCT. A study, where iPSCs were generated from patients with MPS IH (MPS-iPSCs), found that α-L-iduronidase was not required for stem cell renewal, and that MPS-iPSCs showed lysosomal storage characteristic of MPS IH and could be differentiated to both hematopoietic and nonhematopoietic cells (Tolar et al. 2011). The specific epigenetic profile associated with de-differentiation of MPS IH fibroblasts into MPS-iPSCs was maintained when MPS-iPSCs are gene-corrected with virally delivered α-L-iduronidase. Thus MPS-iPSCs can generate autologous hematopoietic grafts devoid of immunologic complications of allogeneic transplantation, in addition to generating nonhematopoietic cells with the potential to treat anatomical sites not fully corrected with HCT.

## *Personalized Gene Therapy of Duchenne Muscular Dystrophy*

Duchenne muscular dystrophy (DMD) is the most common of the various genetic muscular disorders. Treatment is limited to glucocorticoids that have the benefit of prolonging ambulation by ~2 years and preventing scoliosis. Finding a more satisfactory treatment should focus on maintaining long-term efficacy with a minimal side effect profile. Multiple treatment approaches have been tested.

**Table 16.2** Gene therapy approaches to Duchenne muscular dystrophy

| |
|---|
| Viral vector-mediated gene transfer: retrovirus, lentivirus, adenoviral, AAV |
| Nonviral vectors: liposome-mediated gene transfer |
| Plasmid-mediated gene therapy: direct injection of plasmid DNA into the muscles |
| Electrotransfer of naked DNA in the skeletal muscles |
| Liposome-mediated gene transfer |
| Myoblast-mediated gene transfer |
| Repair of the dystrophin gene |
| Antisense approach |
| Pharmacological modulation of dystrophin gene |



Myoblast transfer has been tried for treatment of DMD but the results have not been satisfactory. Stem cells are promising for treating DMD because a small number of cells are required to obtain a therapeutic effect but identification of a stem cell population that provides efficient muscle regeneration is critical for the progression of cell therapy for DMD. However, there are still unanswered questions regarding a variety of stem cells with myogenic potential, numerous cytokines and growth factors acting solo or in an orchestrated manner.

Most attractive are molecular-based therapies that can express the missing dystrophin protein (exon skipping or mutation suppression) or a surrogate gene product (utrophin). Duchenne muscular dystrophy gene that forms the basis of future gene therapy of this disorder, was identified in 1987 (Hoffman et al. 1987). Endogenous gene expression of dystrophin should be restored to >20 % of normal levels for improvement of muscular dystrophy symptoms. It is possible to block expression of both chromosomal copies of the defective native gene by an antisense approach. Normal protein can be expressed by a normal gene construct that is introduced and contains divergent codons to prevent blocking by the antisense compound. Various gene therapy approaches to DMD are shown in Table 16.2.

Other approaches to DMD include increasing the strength of muscles (myostatin inhibitors), reducing muscle fibrosis and decreasing oxidative stress. Additional targets include inhibition of NF-κB to reduce inflammation or promote skeletal muscle blood flow and muscle contractility using phosphodiesterase inhibitors or nitric oxide (NO) donors. The goal of treatment should be to find a product at least as effective as glucocorticoids with a lower side effect profile or with a significant glucocorticoid sparing effect (Malik et al. 2012). Gene therapy still remains the most promising approach. The most promising possibility for the treatment of DMD might be a combination of approaches such as drugs, stem cell and gene therapy.

### Antisense Oligonucleotide-Induced Exon-Skipping for DMD

Exon-skipping, induced by antisense oligonucleotides changes an out-of-frame mutation into an in-frame mutation, aiming at conversion of a severe DMD phenotype into a mild phenotype by restoration of truncated dystrophin expression (Nakamura and Takeda 2009). Many of the mutations associated with DMD can

potentially be rescued by multiple exon-skipping, which employs multiple DNA-like molecules as DNA band-aids to skip over the parts of the mutated gene that block the effective creation of proteins. Synthetic DNA analogs show outstanding stability and sequence specificity yet little or no binding to modulator proteins. An antisense RNA delivered by a retroviral vector in animal models of DMD not only is capable of inhibiting mutant myotonic dystrophy protein kinase transcripts but also can ameliorate dystrophic muscle pathology at the cellular levels. Systemic delivery of the AAV construct results in effective body-wide colonization, significant recovery of the functional properties in vivo, and lower creatine kinase serum levels, suggesting an overall decrease in muscle wasting. The transduced muscles rescue dystrophin expression and display a significant recovery of function toward the normal values at single muscle fiber level. This approach provides rationale for systemic use of AAV-mediated antisense-U1 small nuclear RNA expression for the treatment of DMD.

Development of antisense oligonucleotides with higher stability and lower toxicity, such as morpholinos, has made it possible to restore dystrophin efficiently in dystrophic mice in vivo with no obvious side effects. Efficacy and toxicity of intravenous antisense oligonucleotide (morpholino)-induced exon skipping has been tested in the DMD dog model (Yokota et al. 2009). Weekly or biweekly systemic intravenous injections with a three-morpholino cocktail over the course of 5–22 weeks induced therapeutic levels of dystrophin expression throughout the body, with an average of about 26 % normal levels. This was accompanied by reduced inflammatory signals examined by MRI and histology, improved or stabilized timed running tests, and clinical symptoms. Blood tests indicated no evidence of toxicity. This study provides a proof of concept for systemic multiexon-skipping therapy. By skipping more than a single exon, this so-called DNA band-aid becomes applicable to between 80 % and 90 % DMD patients, including the mutation found in dogs. This study makes exon-skipping as a systemic treatment for DMD in humans a real possibility in the near term. Significant challenges still remain. Successful systemic treatment with morpholinos requires large doses of the antisense molecules and the technology is costly and difficult to obtain. Additionally, treatment in this study showed diminished success at curbing muscle deterioration of the heart, meaning that a more effective and specific delivery system is needed to rescue the organ's delicate tissue in DMD patients.

Exon skipping is not inextricable bound up with splicing regulatory sequences as the binding of an antisense oligoribonucleotide to sequences within the exon is sufficient to induce exon skipping. This implies that probably most exons in the genome are skippable and that exon skipping could be applicable to the majority of mutations, including deletions, duplications, or nonsense mutations in in-frame exons.

Antisense-mediated exon skipping therapy is being developed as personalized therapy for DMD. Drisapersen (Prosensa Therapeutics BV) and eteplirsen, two chemically distinct drug candidates, are currently in clinical development. Their specific physicochemical characteristics each have their advantages and disadvantages with regard to safety and pharmacokinetics. Both candidates demonstrated specific exon 51 skipping to increase muscle dystrophin expression in a mutational subgroup of patients with DMD, and both showed promising effects on the

6MWT. Several candidates designed to skip other exons and address additional mutation groups are currently in preclinical development. However, given the increasingly lower prevalence of mutations, a nonstandard, orphan drug-tailored design of clinical studies is required. This is supported by the encouraging data obtained to date with drisapersen and eteplirsen, and may be based on extrapolation between patient populations, placebo groups and compounds (within a chemical class). A phase I/IIa study was done to assess the safety, pharmacokinetics, and molecular and clinical effects of systemically administered drisapersen and results showed dose-dependent molecular efficacy in DMD patients with a modest improvement in the 6-min walk test after 12 weeks of extended treatment (Goemans et al. 2011). Phase III clinical trials in progress. Such approaches, combined with the ongoing development of new validated endpoints and surrogate biomarkers for DMD, should bring personalized therapy closer for an increasing number of patients (van Deutekom et al. 2013).

## Personalized Treatment of Cystic Fibrosis

Cystic fibrosis (CF) is the most common serious genetic disease among Caucasians in the US. Although a multi-organ disease, CF is usually diagnosed by symptoms of pulmonary infections and mucus plugging of the airways, which result from dysfunction of the CF transmembrane conductance regulator (CFTR), an ion channel that mediates anion transport across epithelia. Definite diagnosis of CF requires proof of CFTR dysfunction, by 'sweat Cl- test'.

More than 10 million Americans are carriers for CF, including 1 in 25 Caucasians. Carrier screening can help physicians identify children with CF earlier in life, allowing parents and medical professionals to begin medical and nutritional intervention that can improve the child's growth and development, and reduce the incidence of respiratory infections. Over 1,000 mutations and DNA sequence variations have been identified in the CFTR gene. The F508 mutation is represented in almost all populations. Carrier testing for CF is aimed at identifying individuals who do not show signs of the disease, but who carry a genetic mutation that can be passed onto their offspring. CF is a potentially lethal disease although the current life expectancy has improved to about 30 years with advances in the medical treatment.

### *Current Management of CF*

Currently used methods for the treatment of pulmonary complications of CF include physiotherapy, bronchodilator therapy, mucolytic agents and corticosteroids. Many drugs, including mucolytics and antibiotics, aim to alleviate the pulmonary symptoms of CF, but do not address the cause of the disease. Lung transplant is the last resort for advanced pathology. Many of these therapies are individualized according to the needs of the patients, which vary considerably.

### *Personalizing New Therapies for CF*

However, new therapies to modulate defective CFTR, the basic defect underlying CF, have started to reach the clinic and several others are in development or in clinical trials. The novelty of these therapies is that, besides targeting the basic defect underlying CF, they are mutation specific. Although a monogenic disease, CF is influenced by a large number of different genes and biological pathways as well as by environmental factors that are difficult to assess. Therefore, every person with CF is unique and functional assessment of patients' tissues ex vivo is important for diagnosis and prediction of severity of this disease and assessment of responses to drugs for effective treatment (Amaral 2014). This is best achieved by a personalized approach to CF.

### *Gene Therapy and Pharmacogenomic Approach to CF*

CF is prime candidate for gene therapy. Pharmacogenomic approach to CF starts with genomic analysis of cells and tissues from CF patients that have been corrected by gene therapy. These serve as end points of successful treatment when studying new drugs candidates for CF. Bioinformatic tools are used to analyze the data and identify genes that reveal drug efficacy. Pharmacogenomic approach may eventually provide the opportunity to create drugs in a patient in a mutation-specific manner.

In 2012, EC approved Vertex Pharmaceuticals' KALYDECO™ (ivacaftor) tablets for the treatment of CF in patients age 6 years and older who have G551D mutation in their CF gene. It is a CFTR potentiator and is not indicated for use in patients with CF due to other mutations in the CF gene. It is not effective in patients with CF with two copies of the F508del mutation (F508del/F508del) in the CF gene.

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# Chapter 17
# Personalized Approaches to Immune Disorders

## Introduction

The innate immune system is the first line of host defense against infectious agents. There are many variations of response in individuals. Immunology has already been playing an important role in personalization of therapy, e.g. blood grouping and cross-matching for blood transfusion.

Comprising the third largest lymphocyte population, natural killer (NK) cells recognize and kill cellular targets and produce pro-inflammatory cytokines. These potentially self-destructive effector functions can be controlled by inhibitory receptors for the polymorphic major histocompatibility complex (MHC) class I molecules that are expressed on target cells. However, the genes for the MHC proteins and the NK cell receptors are inherited independently from one another, and can vary widely. It has been shown that NK cells acquire functional competence through 'licensing' by self-MHC molecules (Kim et al. 2005). This process results in two types of self-tolerant NK cells – licensed or unlicensed – and may provide new insights for exploiting NK cells in immunotherapy. It is possible to engineer entire MHC class I molecules into mouse cells by inserting only that gene. These studies have revealed that developing NK cells are induced to become functional by Ly49 – an inhibitory receptor on their surface, which plays an activating, or licensing, role in enabling immature NK cells to develop into functioning, self-tolerant cells. The licensing concept might explain differences in response among human patients with HCV infections. In many individuals, this virus causes a chronic infection lasting several decades. In other individuals, the virus seems to be controlled and eradicated as they have "better licensed" NK cells that mount a better response to the virus. Licensing might also explain why donor NK cells given to leukemia patients during bone marrow transplantation as treatment do not always have an anti-tumor effect. Although the donor NK cells are expected to attack leukemic cells as being "non-self," the outcome is not as expected in some cases and licensing needs should be considered. Further research is aimed at developing immunological tests to determine if licensing can be used to predict successful eradication of viral infections or anti-leukemia effects.

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Immunological tests have an important place in the future of personalized medicine. The role of immune system in personalization of treatment in infections and cancer has already been discussed in earlier sections.

## Personalized Approaches in Immunology

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Comprising the third largest lymphocyte population, natural killer (NK) cells recognize and kill cellular targets and produce pro-inflammatory cytokines. These potentially self-destructive effector functions can be controlled by inhibitory receptors for the polymorphic major histocompatibility complex (MHC) class I molecules that are expressed on target cells. However, the genes for the MHC proteins and the NK cell receptors are inherited independently from one another, and can vary widely. NK cells acquire functional competence through 'licensing' by self-MHC molecules that results in two types of self-tolerant NK cells – licensed or unlicensed – and may provide opportunities for exploiting NK cells in immunotherapy. NK cells can be induced to become functional by Ly49, an inhibitory receptor on their surface, which plays an activating, or licensing, role in enabling immature NK cells to develop into functioning, self-tolerant cells. The licensing concept might explain differences in response among human patients with HCV infections. In many individuals, this virus causes a chronic infection lasting several decades. In other individuals, the virus seems to be controlled and eradicated as they have "better licensed" NK cells that mount a better response to the virus. Licensing might also explain why donor NK cells given to leukemia patients during bone marrow transplantation as treatment do not always have an anticancer effect. Although the donor NK cells are expected to attack leukemic cells as being "non-self," the outcome in some cases is not as expected and licensing needs should be considered. Further research is aimed at developing immunological tests to determine if licensing can be used to predict successful eradication of viral infections or anti-leukemia effects.

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## Pharmacogenetics and Pharmacogenomics of Immunosuppression

Immunosuppressive therapy has markedly improved over the past years with the advent of highly potent and rationally targeted immunosuppressive agents. Since these drugs are characterized by a narrow therapeutic index, major efforts have

been carried out to define therapeutic windows based on the blood levels of each immunosuppressant, and relating those concentrations to clinical events. Although pharmacokinetic-based approaches are currently used as useful tools to guide drug dosing, they have several limitations. Pharmacogenomics might represent a complementary support. Studies that have focused on polymorphisms of genes encoding enzymes involved in drug metabolism, drug distribution, and pharmacological targets, have shown promising results. Pharmacogenomics holds promise for improvement in the ability to individualize pharmacological therapy based on the patient's genetic profile.

Testing for thiopurine-S-methyltransferase polymorphisms is widely used in clinical practice whereas other pharmacogenetic tests are much less frequently used. Relatively good evidence has emerged for tacrolimus-related biomarkers; thus, their application may be anticipated in the near future. Although the biomarkers related to mycophenolate, sirolimus or other immunosuppressive drugs are promising, further research is required to provide more robust evidence (Hronová et al. 2014).

## Personalized Management of Patients with Lupus Erythematosus

Systemic lupus erythematosus (SLE) affects >1 million persons in the US and Western Europe. It is a chronic B cell mediated disease manifested by arthralgias, fever, skin rash and end-stage renal disease. Although considered a prototypic autoimmune disease, the hallmark of SLE is its heterogeneity. Accordingly, manifestations can vary widely from person to person, with the potential involvement of virtually any bodily organ. Genetic abnormalities underlying this condition are complicated, with diverse genetic polymorphisms described in different ethnic groups, strongly suggesting that the actual pathology underlying the immunologic disarray might not be the same for each patient. There is no cure for this disease.

Only three categories of drugs are currently approved for SLE: corticosteroids, antimalarials, and low-dose aspirin. These are used for symptomatic relief or nonspecific immunosuppression. Until recently, antibodies to dsDNA or nuclear antigens such as Sm antigen and phospholipids or measurement of complement activation were used together with clinical scores as indicators of drug efficacy in clinical trials. Clinical scores are not satisfactory as there is a considerable lag period between initiation of treatment and clinical effects. There are two potential biologic drugs, rituximab (anti-CD20) and anti-CD22, but drug approval agencies are unable to assess their real efficacy because reliable biomarkers are not available.

The lack of reliable, specific biomarkers not only hampers clinical management of SLE but also hinders development of new therapeutic agents. Based on available data, several potential biomarkers for susceptibility, diagnosis, and disease activity have been identified. Despite the complexities of the many immunologic pathways that are involved in SLE, biomarkers are emerging to characterize patient subgroups, predict prognosis, indicate the exacerbations and remissions of SLE flares, and serve as endpoints in the determination of the dosing and timing of immune-modulating

treatments. Several clinical studies have tested new therapies directly targeting B lymphocytes. Flow cytometry of circulating peripheral B lymphocytes have been used to define pathogenic subsets of the disease and assess therapeutic efficacy. Biomarkers for SLE include Fc receptor genes (disease susceptibility), complement C4d-bound erythrocytes (diagnosis or disease activity), CD27 plasma cells (disease activity), 'interferon signature' (disease activity), and anti-C1q antibodies (disease activity and organ involvement). These promising candidate biomarkers need to be validated through rigorous, large-scale multicenter studies. There is still an urgent need for better biomarkers and pharmacodiagnostic tests with which to monitor disease activity in patients with SLE and response to treatment.

Lupus Therasight™ (PIKAMAB) is a proprietary approach to stratify patients based on the FcGR-3A, 2A, 3B, and 2B polymorphisms. These polymorphisms, when collectively correlated, should provide a deeper understanding on the mechanism of onset and progression of lupus and lupus nephritis, and can determine the severity of these diseases in patients irrespective of their ethnicity. The test, which includes a functional assay, can provide significant clues with regard to the progression and severity of these diseases in these patients over a period of time.

## Personalized Therapy of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a multicomplex system inflammatory disorder, which affects the synovial lining of the joints and tendons. As a result of treatment strategies based upon individualized measurement of disease activity, the clinical view of RA has changed from a destructive autoimmune disease to a condition in which significant damage can be prevented in the majority of patients. Although the cause of RA is not known, factors such as genes, epigenetics, environments, local joint characteristics or processes of aging might influence the clinical phenomenon RA (Pieringer and Studnicka-Benke 2013). Environmental factors are generally considered to play a role with systemic immune reactions precipitating a cascade of inflammatory reactions. Consideration of all of these factors is important for planning a personalized approach to management of RA.

### *Genetics and Epigenetic Aspects of Rheumatoid Arthritis*

Hyperproduction of interleukin-6 (IL-6) is observed in RA patients and serum level of IL-6 is closely related to disease activity. IL-6 is a pleiotropic cytokine and its hyperfunctions explain most of the clinical symptoms in RA. Although RA has a complex mode of inheritance, HLA-DRB1 and PTPN22 are well-established susceptibility loci. A common genetic variant at the TRAF1-C5 locus on chromosome 9 is associated with an increased risk of anti-CCP-positive RA (Plenge et al. 2007).

Epigenetics, particularly DNA methylation, is a potential mediator of genetic risk in RA. A study has compared newly diagnosed RA patients and healthy controls,

examining their DNA for chemical tags – methyl groups – that could attach themselves to genes and turn them on or off (Liu et al. 2013). Results showed that the chemical tags may help determine if a person with a gene that increases risk of developing RA actually gets the disease. There were subjects in the control group who had gene variations associated with RA risk, but they did not have those four chemical tags and did not have the disease.

## *Variations in the Effectiveness of Various Treatments of RA*

Numerous drugs are used in the treatment of RA. Some are for relief of pain whereas others are aimed at modifying the disease process. There are large differences in the effectiveness of disease modifying anti – rheumatic drugs (DMARD) from one person to the next. Adverse drug reactions caused by DMARD can also occur in some patients but not in others. Because traditional pharmacotherapy in rheumatology has been empirical and because of the slow acting nature of many anti-rheumatic medications, the risk of significant side effects and the increasing armamentarium of drugs available, pharmacogenetics is particularly relevant to rheumatology. There are many scientific and non-scientific concerns that should be addressed in future studies.

One possible cause of the differences in the effectiveness and adverse drug reactions is genetic variation in how individuals metabolize drugs. Various studies have revealed the relationship between genetic polymorphisms of drug metabolizing enzymes and the efficacy of DMARDs in patients with RA, suggesting pharmacogenetics is applicable to the treatment of rheumatoid arthritis. Methotrexate (MTX) remains the most commonly used disease modifying antirheumatic drug in RA because of its low cost and experience in its use, despite the availability of new treatments such as leflunomide and the anti-cytokine agents. However, a significant number of patients with RA either do not benefit from the drug or are unable to tolerate it. Pharmacogenetic approaches may help optimize treatment with MTX, and also other agents in RA.

Haplotype patterns in the IL-1 gene cluster influence why some individuals respond differently to inflammatory stimuli and thereby develop a different disease pattern or respond differently to therapy. Interleukin Genetics is generating more detailed information on new haplotypes in the IL-1 gene cluster from its high-density SNP mapping project. One of the primary clinical applications that Interleukin is pursuing is the development of a pharmacogenetic test to assist physicians in deciding which therapeutic drugs to prescribe patients with rheumatoid arthritis. Some published data suggest that a patient's IL-1 genotype may predict his or her response to drug therapy.

Pharmacogenomic studies on methotrexate, sulfasalazine and TNF-α inhibitors have been reported, suggesting that the pharmacogenomic approach may be useful for the treatment of RA. Although there other points to be considered before the translation of the pharmacogenomic date into clinical practice, pharmacogenomics is an important tool for development of individualized medicine in the treatment of RA (Taniguchi et al. 2007).

### *Biomarkers for Personalizing Therapy of Rheumatoid Arthritis*

Vectra™ DA (Crescendo Bioscience), multi-biomarker blood test for RA may more accurately identify early RA patients at risk for progression of joint damage when compared to established disease activity measures. Prediction of radiographic progression (RP) in early RA would be very useful for optimal choice among available therapies. The SWEFOT trial evaluated a multi-biomarker disease activity (MBDA) score, based on 12 serum biomarkers as a baseline predictor for 1-year RP in early RA (Hambardzumyan et al. 2014). Results suggest that when choosing initial treatment in early RA the MBDA test may be clinically useful to identify a subgroup of patients at low risk of RP.

Data demonstrate the Vectra DA algorithm score can identify patients at higher risk for structural damage despite achieving remission by DAS28CRP (the 28-joint disease activity score based on C-reactive protein). Among RA patients in DAS28CRP remission, those who also had a high Vectra DA algorithm score are 2.3 times more likely to have progressive joint destruction during the following year. Moreover, patients in remission as defined by the Vectra DA algorithm score have a lower observed rate of radiographic progression compared to patients in remission by DAS28CRP or by the Boolean criteria of American College of Rheumatology and the European League Against Rheumatism.

RA demonstrates a high heterogeneity in clinical responses to treatment. Although the efficacy of biological therapy has undoubtedly been established, the response differs considerably between individuals. This variability between individuals has stimulated search for biomarkers predictive of treatment response. Pharmacogenomics underlying individual responses to drugs is rapidly developed and has the potential of realizing the personalized therapy in RA (Xie et al. 2014).

Patients with RA are generally treated with tumor necrosis factor (TNF)-α inhibitors as second-line therapy if an oral medication such as methotrexate is not adequate to control the symptoms. If one anti-TNF therapy does not lead to adequate symptom control, the current standard of care dictates switching to another approved anti-TNF agent, even though response rates deteriorate with each cycle. Pilot research at Biogen Idec and academic collaborators has developed a panel of gene expression biomarkers with ~90 % positive and negative predictive values to identify individuals who did not achieve European League Against Rheumatism (EULAR) Disease Activity Score (DAS)-28 good response after 14 weeks of treatment. Such a biomarker panel could be used as a diagnostic test to direct therapeutic options.

BATTER-UP (Biomarkers of Anti-TNF-α Therapy Efficacy in Rheumatoid arthritis to define Unresponsive Patients) is a clinical study sponsored by Biogen-Idec for adults diagnosed with RA to predict if a specific person with RA will be helped by anti-TNF-α medications such as Remicade®, Enbrel®, Humira®, Cimzia® and Simponi®. The primary outcome measure will be validation of the ability of an 8-gene biomarker set to differentiate between patients who meet or do not meet EULAR DAS-28 Good response criteria after treatment with anti-TNF therapy. The aim is to develop a test that could help physicians decide whether or not to prescribe an anti-TNF drug for a particular patient.

## *DIATSTAT™ Anti-cyclic Citrullinated Peptides in Rheumatoid Arthritis*

Effective disease management in RA requires early diagnosis and an accurate prediction of which patients will have severe arthritis and require aggressive treatment. There is a need for reliable biomarkers to assist clinical diagnosis and classify patients into erosive and non-erosive forms at the earliest stage. Axis-Shield DIASTAT anti-cyclic citrullinated peptides (CCP) detects antibodies against CPP that are derived from filaggrin, a protein associated with epidermal intermediate filaments. Antibodies to these CCPs correlate positively with the severity and incidence of RA and its symptoms. Anti-CCP shows high sensitivity for RA (50-91 %) versus rheumatoid factor (RF) (70-75 %). Similarly, anti-CCP shows a very high specificity (>97 %) versus RF (<66 %). RF is also present in other autoimmune diseases, infectious diseases and healthy individuals. Anti-CCP in personalized medicine can:

- Detect early onset of RA disease
- Measure severity and erosiveness of RA
- Predict arthritis outcome
- Differentiate between autoimmune diseases
- Stratify RA patients for treatment with disease modifying antirheumatic drugs
- Be used to measure the effectiveness of treatment

## *Personalization of COX-2 Inhibitor Therapy*

COX-2 inhibitors became one of the most widely used drugs for the management of inflammatory pain in rheumatoid arthritis. The best known of these were valdecoxib (Pfizer's BEXTRA), celecoxib (Pfizer's Celebrex) and rofecoxib (Merck's Vioxx). These markedly reduced the gastrointestinal complications of NSAIDs that were used previously for arthritis. However, an increased incidence of cardiovascular complications led to the withdrawal of rofecoxib and restrictions on valdecoxib and celecoxib. Some of the clinical trials for use of COX.2 inhibitors in prevention of cancer and neurodegenerative diseases were also halted. In 2005, a panel of experts voted unanimously to advise FDA that three leading painkillers – Celebrex, Bextra and Vioxx – can cause worrisome heart problems. But it also advised against banning the drugs. There is a potential for application of pharmacogenetic studies to identify patients who are susceptible to cardiovascular complications so that the use of these drugs in such patients can be avoided.

## *Personalization of Infliximab Therapy*

Infliximab, an anti-TNFα antibody, is effective in the treatment of several immunoinflammatory diseases including rheumatoid arthritis. However, many patients experience primary or secondary response failure, suggesting that individualization

of treatment regimens may be beneficial. A study using radioimmunoassays to measure levels of anti-infliximab antibody and of TNFα binding due to infliximab in rheumatoid arthritis patients has shown that development of anti-infliximab antibodies, heralded by low preinfusion serum infliximab levels, is associated with increased risk of infusion reaction and treatment failure (Bendtzen et al. 2006). Early monitoring may help optimize dosing regimens for individual patients, diminish side effects, and prevent prolonged use of inadequate infliximab therapy.

### *Personalized Therapy of RA Guided by Anti-citrullinated Protein Antibodies*

Although a large number of targeted therapies (TNF, IL6, CD80/CD86 and CD20 inhibitors) have become available to better treat the underlying disease process, identification of the underlying pathways that drive the disease process in an individual patient has been relatively unsuccessful, implying that no predictive factors have been identified to guide the choice of a specific treatment. Distinct subsets of RA patients have been identified, based on the presence or absence of anti-citrullinated protein antibodies (ACPAs). Two subsets are associated with different environmental and genetic risk factors, histology and disease outcome. A more destructive disease course with persistent joint inflammation is observed when ACPAs are present. Therefore, treatment should be aimed at a more consistently low level of disease activity in the presence of ACPAs than in the absence of the antibodies (Huizinga 2014).

## Personalized Approaches to Improve Organ Transplantation

Matching in organ transplantation is already personalized. Management of complications, the most important of which is organ rejection, can be improved by personalized approaches. Two examples of typical organ transplants, kidneys and heart, will be used to illustrate how personalized approaches can improve organ transplantation results.

### *Personalization of Kidney Transplantation*

Although tissue and blood matching is done prior to organ transplantation, there are still problems of rejection after transplantation. Among transplant patients, 50 % lose their kidneys within 8-10 years. With immunosuppressants, a transplanted kidney can survive and function well for years. However, immunosuppressants also have a dark side. Immunosuppressive drugs make transplant patients more likely to

suffer heart disease, diabetes, infections and cancer. These drugs are also toxic, and they can slowly poison the very kidney they are protecting. They can also cause hypertension and hyperlipidemia, eventually leading to the failure of the new kidney transplant – a condition known as chronic allograft nephropathy.

Unlike acute rejection, which is entirely the result of the immune system attacking the transplanted organ, chronic allograft nephropathy may be a result of the immune system, the immunosuppressive drugs, or both. It is a major problem in kidney transplantation and >50 % of biopsies taken from kidney transplant patients who appeared to be doing well only 2 years after transplantation already show signs of chronic allograft nephropathy. Serum creatinine, the currently used biomarker to monitor renal transplant patients, is an insensitive, late-trailing indicator of graft function. When creatinine levels are elevated, biopsies are generally performed to assess whether graft function has been compromised and, if so, identify the cause through histological analysis. Biopsies are costly, disruptive, subjective and invasive. They carry the risk of complications and, in one third of the cases, fail to yield useful, actionable information. Gene expression profiling could be used to define a unique molecular signature for chronic allograft nephropathy. Use of this knowledge could help to personalize kidney transplantation and reduce the morbidity.

Transplant Genomics Inc is developing tests that use a broad range of genomic and proteomic tools capable of revealing the complexity of the underlying biology, which is well known to be highly heterogeneous. Compared to conventional methods, these tests will enable earlier detection of graft dysfunction and differential diagnosis among actionable causes, providing an opportunity for physicians to take clinical actions to prolong graft and patient survival. These tests can be used, e.g., in monitoring patients to detect subclinical rejection, deciding when to perform biopsies rather than relying on protocols or waiting for creatinine levels to rise; for optimizing minimization of immunosuppression therapy by ensuring early detection of an immune response; and in molecular profiling of biopsies to complement conventional histology and help resolve ambiguous or borderline cases.

## *Personalization of Cardiac Transplantation*

AlloMap MolecularTesting (CareDx Inc) is a non-invasive gene expression test used to aid in the identification of heart transplant recipients who have a low probability of moderate/severe acute cellular rejection at the time of testing in conjunction with standard clinical assessment. AlloMap testing measures the expression levels of 20 genes from a blood sample. The combined expression of these genes is represented as an AlloMap test score. AlloMap, cleared by the FDA and CE-marked in EU, is performed in the CareDx CLIA-certified laboratory. Use of AlloMap is also included in the International Society for Heart and Lung Transplantation Practice Guidelines, the worldwide standard for the care of heart transplant patients.

AlloMap assays the RNA levels of 11 rejection biomarker genes and 9 control genes, for identification of heart transplant recipients who have a low probability of

moderate/severe acute cellular rejection at the time of testing. IMAGE study in 2010 showed the non-inferiority of clinical outcomes of heart transplant recipients managed with the AlloMap test compared with conventional endomyocardial biopsy. CareDx is exploring the use of cell-free DNA as a biomarker for rejection in heart transplants.

## *Prediction of Rejection for Personalizing Anti-rejection Treatment*

Surgical techniques have improved survival rates for pediatric organ transplantation dramatically over the last 25 years. As a result, the challenge has shifted to improving quality of life. Anti-rejection medications are important because, while they make transplantation possible, but they also can have adverse side effects that can themselves become life-threatening, such as infections and cancers. In order to improve this situation, the NIH awarded a research grant to Children's Hospital of Pittsburgh to study genetic factors that could predispose transplant recipients to rejection. Pre-transplant prediction of which patients are more likely to experience rejection may be used to tailor anti-rejection medications accordingly. Multiple processes that cause rejection in blood cells have been studied and this information will be linked to the unique "genomic fingerprint" of liver transplant candidates, based on the inheritance of >500,000 mutations or SNPs from parent to child. These mutations can be transmitted from parent to child in certain patterns that indicate if a transplant candidate is predisposed to rejection, a rejection-free state or tolerance, a rare occurrence whereby anti-rejection medications no longer are required. Based on the results of this study, a patient more likely to reject a transplanted organ may someday receive high doses of anti-rejection medicine initially. Those who are less likely to reject could have lower doses, or less potent combinations. By applying individualized anti-rejection strategies before the transplant even occurs, the investigators hope to reduce rejection rates and drug-induced side effects for pediatric liver transplant from 50 % to ~20 %.

## *Personalized Immunosuppressant Therapy in Organ Transplants*

Organ transplants are one of the earlier examples of personalized therapy in which organs are matched to the individuals. In spite of this graft-versus-host disease and organ reject remain significant problems. Several immunosuppressent therapies are available now and the responses of individual patients to these vary.

Because of all the drug toxicities, one of the major challenges in treatment following transplant surgery is to determine the proper regimen of immunosuppressant drugs needed for a patient to prevent rejection of the transplanted organ. Patients must be given a strong enough dose of the drugs so that their immune systems are kept in check. At the same time, they cannot receive so high a dose that the drugs are toxic to the new kidneys. Balancing the need for more with the need for less is made more difficult by the fact that every patient responds differently to the immunosuppressant drugs.

Several novel immunosuppressive agents and new formulations, including sirolimus, mycophenolic acid (the active metabolite of mycophenolate mofetil), tacrolimus, and microemulsion cyclosporine, have significantly improved the clinical outcome of transplant recipients. However, the majority of immunosuppressive agents need a constant monitoring of drug levels to reduce the risk of graft rejection as well as drug-induced toxicities. Many factors may affect the pharmacokinetic characteristics of immunosuppressive agents, potentially reducing treatment effectiveness. Absorption and metabolism of immunosuppressive drugs are influenced by patient genotype and comedications, while comorbidities (i.e., diabetes and cystic fibrosis) are responsible for altered pharmacokinetics. There are a number of associations between genotype and pharmacology and donor genotype may play a significant role in immunosuppressive drug pharmacokinetics and pharmacodynamics (Fu Liang et al. 2007). Dose individualization in transplant recipients is performed according to their health status, graft function, and drug therapeutic range. Therapeutic drug monitoring plays a crucial role in achieving optimal immunosuppression, improving the efficacy of drugs, and lowering toxic effects. Recent studies have investigated treatment individualization by evaluating drug pharmacogenetics based on the expression level or mutations of their molecular targets, including calcineurin for cyclosporine and tacrolimus, and inosine monophosphate dehydrogenase for mycophenolic acid. Although no conclusions can be drawn from the data of preliminary trials, further studies are underway to address the role of pharmacogenetics in clinical decision making for immunosuppression.

Pharmacogenomics can be used to match patients to immunosuppressants. The discoveries of genomic science can be used to build a new set of tools so that doctors can measure and predict how a patient will respond to immunosuppressive drugs. With such tools, transplant physicians could monitor patients regularly to make sure their treatment is always optimal. In fact, these same tools could also guide therapy of patients with diabetes, systemic lupus, rheumatoid arthritis and other immune-related diseases. The basis of this approach is that there may be some genetic "signature" within donors and recipients that predict the best course of treatment following a transplant surgery. This signature could be within the tissues of the transplanted organ or in the blood cells. An example of application of personalization of immunosuppression is kidney transplantation.

## *Role of Immunological Biomarkers in Monitoring Grafted Patients*

Following transplantation of major organs such as heart, kidney, and liver, rejection of grafted organs is an important problem. There is a need for non-invasive tests to monitor these patients for adjusting their immunosuppressive drug treatment and early detection of rejection. There is a need for discovery of predictive biomarkers for these patients.

Gene expression signatures have been studied in peripheral blood mononuclear cells isolated from patients with autoimmune GvHD and immunosuppressed

transplant recipients. A sentinel signature has been characterized raising the possibility of application of blood leukocyte expression signatures for assessment of immune status and early detection of disease.

According to the NIH consensus development project on criteria for clinical trials in chronic GVHD, the following applications of biomarkers could be useful:

- Predicting response to therapy.
- Measuring disease activity and distinguishing irreversible damage from continued disease activity.
- Predicting the risk of developing chronic GVHD.
- Diagnosing chronic GVHD.
- Predicting the prognosis of chronic GVHD.
- Evaluating the balance between GVHD and graft-versus-leukemia effects (graft-versus-leukemia or GVT)
- Serving as a surrogate end point for therapeutic response.

With the advancement of many high-throughput 'omics techniques such as genomics, proteomics, and metabolomics, efforts have been made to understand potential mechanisms of specific graft injuries and develop novel biomarkers for acute as well as chronic rejection (Sarwal 2009). Microarrays are being increasingly used to identify specific patterns of gene expression that predict and characterize acute and chronic rejection, and to improve the understanding of the mechanisms underlying organ allograft dysfunction. It is feasible to develop minimally invasive, rapid tests for prognosis and diagnosis in personalized management of transplantation patients.

Rashes are very common in patients after bone marrow transplants. They may signal the onset of acute GVHD. But until now, a skin biopsy was the only reliable way for doctors to determine whether the rash is caused by antibiotics commonly used to treat bone marrow transplant patients, or is instead GVHD of the skin, where the disease appears in about half of cases. Quantitative proteomic studies have shown that elafin is overexpressed in GVHD skin biopsies and plasma concentrations of elafin are significantly higher at the onset of skin GVHD (Paczesny et al. 2010). These are correlated with the eventual maximum grade of GVHD, and are associated with a greater risk of death relative to other known risk factors. Therefore, elafin has significant diagnostic and prognostic value as a biomarker of skin GVHD. The test, which is available to clinicians, can determine the risk a patient may have for further complications, and thus physicians will be able to adjust therapy to the degree of risk, rather than treating every patient in exactly the same way.

A study compared 12 biomarkers in plasma obtained a median of 16 days after therapy initiation from patients with a complete response by day 28 after therapy initiation and in plasma obtained from patients with progressive GVHD during therapy (Vander Lugt et al. 2013). The lead biomarker, suppression of tumorigenicity 2 (ST2), had the most significant association with resistance to GVHD therapy and subsequent death without relapse. As compared with patients with low ST2 values at therapy initiation, patients with high ST2 values were 2.3 times as likely to have treatment-resistant GVHD and 3.7 times as likely to die within 6 m after therapy.

Patients with low ST2 values had lower mortality without relapse than patients with high ST2 values, regardless of the GVHD grade. Plasma ST2 values at day 14 after transplantation were associated with 6 m mortality without relapse, regardless of the intensity of the conditioning regimen. ST2 levels measured at the initiation of therapy for GVHD and during the first month after transplantation improved risk stratification for treatment-resistant GVHD and death without relapse after transplantation.

## Improved Matching of Blood Transfusion

Blood transfusions are among the earliest forms of personalized therapies because the blood groups of the donor and recipient are matched. Whilst blood transfusions are inherently safe with the compatibility between the donor and the recipient being tested using serological techniques, there is a significant section of the population that suffer serious illness and side effects after receiving multiple transfusions of blood that is not a perfect match. These patients develop antibodies after some time that reject imperfectly matched blood transfusions, a process known as alloimmunization, which can lead to serious illness and life-threatening side effects.

Bloodchip will provide the medical community with a much clearer picture of the many different and often small variations in blood types, thereby allowing more accurate matching of donors and recipients. The new test will be of real benefit to patients who currently receive multiple blood transfusions and require a perfect match in blood types. Bloodchip has been developed by the Bloodgen Consortium, a pan-European group of academic institutions, national blood transfusions services in the UK, Germany, Sweden, Spain, the Czech Republic and the Netherlands, and will be manufactured by Progenika Biopharma. The Bloodchip test will literally be a life saver for those who suffer from illnesses that require multiple blood transfusions such as hemophilia, sickle cell disease and thalassemias by ensuring that the patients receive perfectly matched blood to enable them to better manage their conditions. Bloodchip has already been tested on 3,000 patients with the results compared against the traditional serological test and will shortly be awarded the European CE mark and undergo intensive clinical trials. Bloodchip has been widely accepted by the medical community and will become the new standard for the testing of blood types in course of time.

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# Chapter 18
# Personalized Approaches to Miscellaneous Problems in Healthcare

## Personalized Management of Diabetes

Worlwide prevalence of diabetes mellitus is ~347 million. There are two main types: type 1 diabetes mellitus (T1DM) or insulin-dependent DM affecting 10 % of individuals and type II diabetes mellitus (T2DM) affecting the rest, i.e., 90 %. Monitoring of diabetes mellitus is rapidly advancing toward fully automated glucose control systems such a personalized glucose advisory system (PGASystem) for management of DM. Adults with T1DM appear to be enthusiastic about using a PGASystem system for their diabetes management but also have significant concerns affecting their overall willingness to follow such a system's advice because of the following concerns: (1) how the advice is generated; (2) relinquishing control to automated technology; and (3) inadequate personalization of the system (Shepard et al. 2012).

DM provides an example of chronic disease management with a particular focus on patient self-management. Despite advances in DM therapy, many affected persons still fail to achieve treatment targets and remain at risk of complications. Personalizing the management of diabetes according to the patient's individual profile can help in improving therapy adherence and treatment outcomes. A 6-step cycle for personalized DM (self-) management and collaborative use of structured blood glucose data has been described (Ceriello et al. 2012). E-health solutions can be used to improve process efficiencies and enable remote access. Decision support tools and algorithms can help physicians in making therapeutic decisions based on individual patient profiles.

There is a need for technology that can accurately assess β cell death in order to improve diagnosis of DM, allow for disease staging, and provide improved evaluation of the efficacy of treatment. A PCR-based technology (Islet Sciences) can be used to identify β cell death before the onset of hyperglycemia and soon after the onset of T1DM. The method uses a stepwise detection and analysis of β cell and non-β cell-derived insulin DNA based on the existence of unique DNA methylation patterns in the β cells that are absent from other cells in the body.

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Efforts to prevent DM in the future will be tailored to high-risk individuals rather than populations and will be based on genetic and other new biomarker tests. Accurate biomarker tests to identify people at risk for diabetes could enable targeted and individualized prevention efforts. DNA variants conferring higher risk for T2DM have been identified, but these account for only a small fraction of genetic risk, which limits their practical predictive value (Spiegel and Hawkins 2012). Identification of these variants has not yet led to new, individualized prevention methods. Further research is needed to identify genomic and other types of biomarkers that could accurately predict risk and facilitate targeted prevention.

T2DM is commonly treated with more than one type of therapy, including oral antidiabetic drugs (OADs) and agents used in the treatment of diabetic complications. Several pharmacological classes of OADs are currently available for the treatment of T2DM, of which insulin secretagogues (i.e. sulphonylureas and meglitinides), insulin sensitizers (thiazolidinediones) and biguanides are the most commonly prescribed. Although many of these OADs have been used for more than half a century in the treatment of T2DM, the pharmacogenomic characteristics of these compounds have only recently been investigated, primarily in retrospective studies. Advances in pharmacogenomics have led to the identification of polymorphisms that affect the expression and function of drug-metabolizing enzymes and drug transporters, as well as drug targets and receptors. Pharmacogenomic data obtained from studies of T2DM treatment, with a focus on polymorphisms in genes affecting pharmacokinetics, pharmacodynamics and treatment outcome of the most commonly prescribed OADs throws some light on the therapeutic response to and side effects associated with OADs (Emami-Riedmaier et al. 2014). Novel ‘omics’ technologies and might aid in the personalized management of T2DM.

## Personalized Management of Gastrointestinal Disorders

### *Inflammatory Bowel Disease*

Inflammatory bowel disease (IBD) refers primarily to two diseases – ulcerative colitis and Crohn’s disease – but the cause remains unknown. The incidence and prevalence of IBD varies widely throughout the world; they are considerably higher in the US and Europe than in Asia and Africa. Most studies indicate a range of 4-8 new cases per 100,000 population per year in the US and Europe. IBD patients are treated by sulfonamides, steroids and immunosuppressants. For difficult cases, leukocytapheresis, beclomethasone dipropionate, anticytokines and other new therapies are tried.

IBD First Step SM and IBD Diagnostic System (Prometheus Laboratories) have the potential to decrease the number of diagnostic procedures (including colonoscopies and radiographs) currently used to identify and subtype IBD from non-IBD disorders. Imuran immunosuppressive therapy can be optimized with PRO-PredictRx (Prometheus Laboratories).

### Biomarkers of IBD

In 2012, Genisphere and the Lankenau Institute for Medical Research expanded their research collaboration covering miRNA biomarkers for ulcerative colitis to include Crohn's disease and other IBDs. The partners will use the Affymetrix GeneChip miRNA Array to identify the miRNA biomarkers. The goal is to identify biomarker panels that can classify the various forms of IBD and develop a diagnostic test to shed light on how patients are being correctly or incorrectly diagnosed in their respective diseases and to provide information on how they are responding to treatment. This will enable personalized treatment of IBD.

Chromosome 16 and the HLA region on chromosome 6 have been implicated in susceptibility to Crohn's disease. Mutations in the NOD2/CARD15 gene, identified on chromosome 16, have been associated with Crohn's disease overall, but are found in only 25 % of patients. The clinical pattern of Crohn's disease may be defined by specific genotypes. Advancement of genome analysis might have an impact on the treatment of inflammatory bowel diseases. Genomic studies have revealed some genetic factors contribute to pathogenesis of IBD such as HLA, IL4, MUC3, IBD1 locus, IBD2 locus. More information about genes concerning IBD will be provided by analyzing dense SNP map using DNA tip. They will open the way to personalized therapy of IBD.

### Personalized Approaches to Management of IBD

There are few proven examples of the importance of pharmacogenetics of serotonin-modifying agents used in functional gastrointestinal or motility disorders. Genetic variations in transporters and translation mechanisms have been associated with responses to treatment in IBD (Camilleri 2007). Research on the impact of polymorphisms of key proteins on the pharmacokinetics and pharmacodynamics of drugs that alter serotonin-mediated signaling will assist in explaining diverse responses to those drugs and ultimately improve personalized approach to IBD.

Personalized approach to management of IBD is evolving along with development of new therapies. MAbs directed against TNF, in combination with immunosuppressive drugs, are effective in high-risk patients if initiated early in the course of IBD. Therapy of IBD can be optimized by appropriate pretreatment testing and patient-based monitoring strategies (Mosli et al. 2014). Although most patients respond to ant-TNF therapy, some do not respond or lose response to the drug over time, which can be caused by patient, TNF-inhibitor, or disease-related factors influencing the pharmacokinetics and pharmacodynamics of the drug. Therefore, target concentration adjusted dosing by therapeutic drug monitoring, may help to guide therapeutic decisions in line with concepts of personalized medicine (Vande Casteele et al. 2014). Algorithms for the management of patients with IBD require a personalized approach incorporating likely natural course of the disease in the individual patient, a strategy for management of loss of response to current therapies, and probability of response to a specific therapeutic agent (Ananthakrishnan 2013).

### *Personalized Management of Lactose Intolerance*

Lactose intolerance is usually due to insufficient lactase and the patient is unable to break down lactose, the predominant sugar found in milk and other dairy products. This results in lactose intolerance symptoms such as nausea, cramps, bloating, gas and diarrhea. Between 30 and 50 million Americans are lactose intolerant. Currently, no treatment exists to improve the body's ability to produce lactase, but symptoms can be controlled through diet and lactase enzyme supplements.

Many other diseases, such as irritable bowel disease and celiac disease, can present with these same symptoms. Improperly diagnosed and unmanaged, these diseases can lead to serious complications. Until now, diagnostic methods used to detect lactose intolerance could not determine the underlying cause, making it difficult for physicians to customize critical patient treatment. A highly-specific, proprietary genetic test, PRO-GenoLogix Lactose Intolerance (Prometheus Inc), identifies patients with a certain genetic biomarker that is associated with lower than normal levels of the lactase enzyme. This genetic test will be especially helpful in differentiating genetic lactose intolerance from other diseases with overlapping symptoms thus eliminating confusion in the diagnostic work-up and therapeutic plan. In addition, this simple blood test does not require patients to undergo fasting, dietary restrictions or lengthy sample collection and, therefore, will likely be better tolerated by patients. The results of this test will enable physicians to individualize treatment of their patients by discerning whether a patient has a genetic basis for lactose intolerance or if their symptoms are related to another disease or disorder.

## Personalized Geriatrics

Geriatrics, the branch of medicine dealing with disorders of elderly, is a recognized sub-specialty. There is no separate chapter on geriatrics in this book as many of the diseases described in various chapters of this book occur at various ages from infancy to old age although some occur more commonly in the elderly. This section will point out some issues that should be taken in consideration in personalized management of the elderly patients.

Prevalence of both therapeutic failures and adverse drug reactions are significantly higher in older subjects. This might be due to higher incidence of polypharmacy and multiple co-existing diseases. Nevertheless, other explanations must also be sought. There are alterations in metabolism and pharmacokinetics due to impairment of renal and hepatic functions that are common in the elderly.

### *Chronological vs Biological Age*

In conventional medicine, most of the physiological parameters and laboratory values are based on chronological age of the patient. An elderly patient undergoing pulmonary or cardiovascular investigation that slight impairment of performance is

still within the norm for his or age whereas prior to illness, the performance might have been >50 % as compared to average persons of his age. People age at different rates depending on several factors including genetic, environmental and life style. A physical active 70-year old may have been performing at the level of a 50-year old prior to onset of disease. In spite of slight impairment of function, his performance may still be within the normal range for his chronological age but may indicate early disease. This factor may be overlooked by the physician but a personalized approach takes this into consideration as a person is his or her own control even within the span of time.

### *Pharmacogenetics and Adverse Drug Reactions*

Prevalence of both therapeutic failures and adverse drug reactions are significantly higher in older than in younger subjects. This might be due to higher use of polypharmacy and multiple co-existing diseases in the elderly. Nevertheless, other explanations must also be sought. There are alterations in metabolism and pharmacokinetics due to impairment of renal and hepatic functions that are common in the elderly. Pharmacogenetics of drug metabolizing enzymes, drug transporters and receptors should not be overlooked.

## Personalized Management of Skin Disorders

There is an overlap between cosmetics, skin care and therapy of skin disorders. Everything from ancient herbs to sheep placentas has been used to make skin care products.

### *Genetic Testing for Personalized Skin Care*

Lab 21 (New York) claims that by taking DNA samples from customers it can provide a personalized skin cream based on specific variations of five genes related to skin sensitivity and aging. The only way to get the formula is to visit one of the company's shops. After answering a 10-min online questionnaire about their skin, ethnic origins, pore size and hydration, the customers get the inside of their mouths swabbed for a DNA sample. The test and the sample are sent to a laboratory to be analyzed and the customized skin creams are generated based on the results. Some geneticists and dermatologists are rather skeptical about this product. It is not a product that is genetically programmed for their skin. Simply studying a DNA sample, without the knowledge of genes that regulate skin health is unscientific. Another issue is privacy because the swabs taken at the shops contain a complete set of an individual's genetic information including genes relevant to several diseases.

Lab 21 says they'll keep all genetic information private, and their Web site claims the genetic samples are destroyed immediately after the analysis is complete.

GeneLink Inc invented the first genetically designed patentable DNA test for customized skin care products, and in partnership with DNAPrint, is screening millions of candidate biomarkers. Tests are designed to assess genetic risks for certain skin disorders due to nutritional deficiencies and provide a basis for recommending formulations that have been specifically designed to compensate for these deficiencies.

### *Management of Hair Loss Based on Genetic Testing*

Androgenetic alopecia occurs with increasing phenotypic expression based on advancing age, approximately 65 % men and 50 % of women will be affected by the age of 60. Clinical diagnosis relies largely on the development of a hair loss pattern, and visible areas of thinning or baldness, which is not apparent until approximately 50 % of hair are lost in a given area. Thus patients will have substantial hair loss prior to initiation of therapy. However, the two FDA-approved medications to combat hair loss, minoxidil and finasteride, are most effective at stabilizing hair loss rather than hair regrowth. Therefore, a screening test for androgenetic alopecia which identifies patients at higher risk for developing it can offer the opportunity for early medical intervention prior to visible signs of hair loss.

An association between male pattern baldness and the androgen receptor gene has been confirmed (Levy-Nissenbaum et al. 2005). HairDX (www.hairdx.com) provides genetic tests for both male and female androgenetic alopecia, which are administered in the privacy of a physician's office using a simple cheek swab. HairDX (RxR) genetic test for finasteride response provides men with a score – CAG repeat score. Smaller CAG test score is associated with an increased response to finasteride for treatment of androgenetic alopecia in men. Among men that had the best response to finasteride approximately 70 % had a CAG score below 22 while among men that had a subtle response to finasteride approximately 70 % had a CAG score above 22. This test helps to personalize treatment of androgenetic alopecia.

## Personalized Preventive Medicine

Genomics and genetics are vital for the development of preventive medicine. Current practice of preventive healthcare involves general advice applicable to population at large, e.g. dietary measures to lower cholesterol. Integration of new genetic information into epidemiologic studies can help clarify causal relations between both life-style and genetic factors and risks of disease. An example is prevention of atherosclerosis where multiple factors interplay in the etiology. Since atherosclerosis involves arterial inflammation, a polymorphism in the 5-lipoxygenase gene promoter could relate to atherosclerosis in humans and that this effect could interact with the dietary intake of competing 5-lipoxygenase substrates.

Inflammatory mediators, leukotrienes, are generated from arachidonic acid (polyunsaturated n-6 fatty acid) by the enzyme 5-lipoxygenase. Variant 5-lipoxygenase genotypes have been found in persons with increased atherosclerosis suggesting that dietary n-6 polyunsaturated fatty acids promote, whereas marine n-3 fatty acids inhibit, leukotriene-mediated inflammation that leads to atherosclerosis in these persons. Such findings could lead to new dietary and targeted molecular approaches for the prevention and treatment of cardiovascular disease according to genotype, particularly in populations of non-European descent.

The significance of risk factors and measures to counteract them vary considerably from one individual to another. General advice to a person to modify all risk factors may not be practical and the compliance is usually low. By identifying genetic predisposition to disease, the physician could focus on risk assessment and develop a comprehensive personalized plan to modify risk factors, and initiate preventive strategies. A practical scenario in preventive medicine practice could be as follows:

A buccal smear sample can be taken in the physician's office for DNA analysis and analysis may eventually be performed for a very reasonable cost to provide information about predisposition to specific diseases. The physician can use this information and draw up a personalized prevention plan taking into consideration the life style of the individual.

## Female Sexual Dysfunction

Female sexual dysfunction (FSD) is the broad term covering a number of disorders from menarche to menopause, which result from an interaction of psychosocial and biological factors modulating the expression of sexual symptoms and associated distress. These are dependent on genetic and epigenetic mechanisms, including acquired medical conditions. Personalized management of FSDs requires an understanding of psychological and environmental determinants as well as the genetic basis to select the most effective intervention for an individual. However, there is a paucity of studies of genetic contribution to FSD. and pharmacogenomics is still in its infancy in the field of sexual medicine as most of the data regarding genetic polymorphisms of drug targets associated with susceptibility to sexual dysfunction have been obtained in males. There is a need for pharmacogenomic studies of FSDs to guide an individualized approach by predicting both therapeutic effects at varying dosages of hormonal and nonhormonal agents as well as, adverse drug reactions and drug interactions (Nappi and Domoney 2013).

### *Hormone Replacement Therapy in Women*

There is some controversy about the usefulness and risks of hormone replacement therapy (HRT) in postmenopausal women.

Sequence variants in the gene encoding estrogen receptor alpha (ER-α) may modify the effects of hormone-replacement therapy on levels of high-density lipoprotein (HDL) cholesterol and other outcomes related to estrogen treatment in postmenopausal women. Some clinical trials have shown that postmenopausal women with coronary disease, who have the ER-alpha IVS1-401 C/C genotype, or several other closely related genotypes, have an augmented response of HDL cholesterol to hormone-replacement therapy. These point to the possibility of using genetic screening for tailoring decisions about hormone-replacement therapy for maximizing the health and wellbeing of postmenopausal women. It is conceivable that, ultimately, more comprehensive pharmacogenomic studies of HRT, in conjunction with more detailed phenotypic markers of disease outcome will lead to effective algorithms for individualizing HRT for postmenopausal women.

## Personalized Management of Osteoporosis

Osteoporosis, a disease characterized by reduced bone mass and increased skeletal fragility, affects 10 million Americans; another 34 million are at risk for it. Because of a large number of causes as well as risk factors, there are wide variations in course of osteoporosis and response to treatment. Calcium and vitamin D is used commonly for prevention of osteoporosis in those at risk, e.g. postmenopausal women. Once considered to be an inevitable consequence of aging, osteoporosis is both diagnosable and treatable.

Bisphosphonates are widely prescribed for treatment of osteoporosis. Examples include: alendronate (Fosamax), risedronate (Actonel, Atelvia), ibandronate (Boniva), and zoledronic acid (Reclast, Zometa). All the bisphosphonates that have been approved for the treatment of osteoporosis have shown robust efficacy in preventing fractures in clinical trials lasting 3–4 years, but data on safety have raised concern regarding the optimal duration of use for achieving and maintaining protection against fractures. Current FDA labeling states: “The optimal duration of use has not been determined. All patients on bisphosphonate therapy should have the need for continued therapy re-evaluated on a periodic basis.” To optimize the efficacy of bisphosphonates in reducing fracture risk, decisions to continue treatment must be based on individual assessment of risks and benefits. In this regard, patients at low risk for fracture (e.g. younger patients without a fracture history and with a bone mineral density approaching normal) may prove to be good candidates for discontinuation of bisphosphonate therapy after 3–5 years, whereas patients at increased risk for fracture (e.g. older patients with a history of fracture and a bone mineral density remaining in the osteoporotic range) may benefit further from continued bisphosphonate therapy. Further investigation into the benefits and risks of long-term therapy, as well as surveillance of fracture risk after discontinuation of bisphosphonate therapy, will be crucial for determining the best regimen of treatment for individual patients with osteoporosis (Whitaker et al. 2012).

## Personalized Management of Renal Disease

Angiotensin converting enzyme (ACE) inhibitors preserve native kidney function in patients with renal disease better than other antihypertensive drugs, most likely because they more effectively reduce proteinuria. The plasma concentration of the ACE inhibitors target is, at least in part, under genetic control. A polymorphism of the ACE gene based on the presence or absence of a 287 base pair element in intron 16 accounts for 47 % of the total phenotypic variance in the plasma ACE levels of healthy individuals. Polymorphisms of the ACE gene account for half the variance in ACE levels in Caucasian but not in Black individuals. Unfortunately, pharmacogenetic studies performed so far do not provide a clear answer as to whether the efficacy of the reduction of proteinuria by ACE inhibitors is influenced by the ACE genotype – probably because these studies were not primarily designed to answer this question. Pharmacogenomics of the ACE inhibitors needs to be examined in a properly designed pharmacogenomic study with a defined endpoint and an appropriately selected control population.

A personalized approach has been applied to the management of type I primary hyperoxaluria an inherited kidney disorder that can cause organ failure in children and young adults. Early diagnosis is important, as the condition, if not treated early and correctly, can cause kidney stones or kidney failure in half of the patients and necessitate a transplant. A genetic mutation (c.508) allows certain kidney stone patients to benefit from vitamin B6 and this finding has been used to develop a genetic test to predict which patients are best suited for this treatment. The gene defect responsible for the disorder disrupts production of a key enzyme, alanine:glyoxylate aminotransferase, located in the liver. The enzymatic deficit causes the liver to produce too much oxalate, which is excreted in the urine. High concentrations of oxalate in the urine can cause kidney stones and injury to the kidney, leading to kidney failure.

## Personalized Care of Trauma Patients

Traumatic injuries claim hundreds of thousands of lives each year in the US. In addition, millions of patients are hospitalized, at an annual cost to society of more than $200 billion. Patients may face a long and difficult recovery period riddled with many potentially fatal complications along the way.

It is important to understand the genetic features that enhance a patient's recovery as well as the elements that cause people to die sometimes weeks after an injury occurs. Identifying those factors could help physicians choose the best treatment, a decision that could mean the difference between life and death. Although most of the trauma patients recover, a fraction develop complications that lead to infection and multisystem organ failure, which is the most common cause of death after traumatic injury. The goal is to use functional genomics as a tool to identify those patients who, after severe trauma and burn injury, will go on to manifest multisystem organ failure.

A genetic tool with the potential to identify trauma and burn patients that are most likely to become seriously ill has been tested in a wide range of experimental clinical settings using blood and tissue samples (Cobb et al. 2005). The authors correlated molecular markers with white blood cell behavior, and ultimately, with patient outcome. They were able to consistently analyze which genes are active in patients with serious infections or traumatic injuries. The major source of variance in apparent gene expression in the blood compartment was found to be due to interindividual variance and not analytical noise. The results reveal a notably high degree of reproducibility both with the analytical processes and in the same subject. The magnitude of the interindividual variance and the changes in gene expression produced by traumatic injury were somewhat greater than the variance associated with the sample processing and analysis in the same subject.

However, prior to adopting this approach in clinical practice, it will be necessary to continue the experimental procedures in larger multicenter trials, following hundreds of patients over time to describe the molecular profile of healing in response to burns and traumatic injury.

## Personalized Medical Care of Astronauts During Space Flights

These differences among astronauts, as revealed by "Omics" technologies, can be amplified in extreme conditions, such as space flight. A better understanding of individual differences may enable development of personalized countermeasure packages that optimize the safety and performance of each astronaut. "Omics" will enhance our ability to: (1) more thoroughly describe the biological response of humans in space; (2) describe molecular attributes of individual astronauts that alter the risk profile prior to entering the space environment; (3) deploy Omics techniques in the development of personalized countermeasures; and (4) develop a comprehensive Omics-based assessment and countermeasure platform that will guide human space flight in the future (Schmidt and Goodwin 2013). Selected examples where biochemical individuality might significantly impact countermeasure development include gene and small molecule variants associated with: (1) metabolism of therapeutic drugs used in space; (2) one carbon metabolism and DNA stability; (3) iron metabolism, oxidative stress and damage, and DNA stability; and (4) essential input (Mg and Zn) effects on DNA repair. Omics profiling should serve as the basis for research in aerospace personalized medicine and explore methodological considerations to advance the field. Personalized medicine may become the standard of care for humans in space in the future.

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# Chapter 19
# Personalized Non-pharmacological Therapies

## Introduction

Most of the discussion in personalized medicine relates to pharmacological therapies. Some of the complementary therapies such as acupuncture do not use drugs. Some non-pharmacological approaches that have become a part of integrated modern healthcare are also personalized and will be discussed here briefly. There are personalized aspects of surgery as well.

## Acupuncture

Acupuncture, as the derivation of the word implies (acus meaning needle; puncta meaning puncture), is the insertion of a needle into the skin of the human body. The ancient Chinese attributed disease to an imbalance between Yin (negative) and Yang (positive) forces. Acupuncture was supposed to restore the balance between these two forces. Acupuncture was used mostly for the relief of pain and muscular disability but has been applied to other disorders as well. The mechanism of action is not well understood and is the topic of most of the research studies.

Acupuncture is the most commonly integrated of the alternative methods into conventional medical practice. It does not conflict with modern neuromuscular anatomy and pain physiology even though it is based on the classical Chinese concept of a subtle circulation network of a vivifying force called Qi. This hybrid acupuncture approach expresses the best of both worlds by describing a context in which to organize patient symptoms that usually escape attention in the standard medical evaluation.

Acupuncture is performed at certain specified points (acupuncture points) that are located on the 12 major meridians on the body, each corresponding to a major organ system of the body. More than 1,000 such points exist; they have been determined by trial and error. Computers are used in acupuncture, and computerized

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manikins are available as a guide for location of acupuncture points corresponding to certain symptoms. There is no standard protocol that is universally applied for all patients. The selection of acupuncture points is personalized according to each patient, location of pain and other symptoms.

### *Personalized Acupuncture Therapy*

Acupuncture needs to be tailored to each patient's particular symptoms as well as responsiveness and it is generally recognized that individualization of acupuncture treatment enhances its effectiveness. Apart from variable selection of acupuncture points, the dose should be determined for each patient. The dose depends not only on the intensity of stimulation but may be affected by the state of the patient, e.g., nervous, immune and endocrine systems; different doses may be required for different conditions (White et al. 2008).

A randomized controlled trial has compared the effectiveness of standardized and individualized acupuncture treatment in patients with chronic low back pain (Pach et al. 2013). Patients received either standardized acupuncture or individualized acupuncture. Treatment consisted of 10–15 treatments based on individual symptoms with two treatments per week. The main outcome measure was the area under the curve (AUC) summarizing 8 weeks of daily rated pain severity measured with a visual analog scale. No significant differences between groups were observed AUC between individualized acupuncture and standardized acupuncture. The authors suggested that a multicenter noninferiority study should be performed to investigate whether standardized acupuncture treatment for chronic low back pain might be applicable in a broader usual care setting.

## Personalized Hyperbaric Oxygen Therapy

Hyperbaric oxygenation (HBO) involves the use of 100 % oxygen under pressure greater than that found on earth's surface at sea level and has proven useful in treatment of several disorders (Jain 2009). The treatments are administered in a hyperbaric chamber. Although there are guidelines regarding pressures and durations of exposure to HBO, patient responses vary. Most of the conditions require repeat sessions of treatment. Parameters of HBO application can be adjusted depending on response to initial treatment. Responses may be assessed clinically but responses to CNS disorders can be evaluated objectively by molecular imaging techniques. Various technologies available for this purpose are positron emission tomography (PET), single-photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI). MRI is used to assess the effect of HBO on multiple sclerosis lesions in the brain. 18F-fluorodeoxyglucose (FDG)-PET is used for determining cerebral blood flow (CBF) and metabolism. The use of PET is limited by a high

cost, the need for a nearby cyclotron to produce radioisotopes with short half-lives. Routine use for monitoring HBO therapy is not currently practical. It is extremely sensitive in the early detection of a cerebrovascular disturbance and can delineate the natural course of an episode that can lead to cerebral infarction. Evidence of ischemia is clearly demonstrated by substantial reduction in CBF and elevated CMR O2 and CMR glucose. The effect of a therapeutic intervention can be assessed by demonstrating the complete or partial reversal of these physiological and biochemical parameters.

SPECT is a useful tool for assessing the effect of HBO in neurological disorders. It is based on principles similar to those of PET but the radioligands decay to emit only a single photon. Advantages of SPECT over PET are:

1. It is more widely available and less costly than PET scan.
2. Any nuclear medicine facility with a gamma camera has the capability for this procedure.
3. There is a short waiting period for uptake of the isotope.
4. The procedure can be integrated with HBO sessions and a post-HBO scan can be done with the same injection as for the pre-HBO scan.
5. This scan documents the area of cerebral infarction as diminished uptake, and any improvement is easy to document by noting the increased uptake of the tracer.
6. Improvement in the scan can be correlated with clinical improvement.
7. SPECT performed within 24 h may be helpful in predicting outcome in clinical practice and in appropriately categorizing patients into subgroups for clinical trials.

If neurologic deficits improve transiently following a treatment and recur after the effects of HBO wear off and this phenomenon can be shown repeatedly, then it can be considered proof of the efficacy of the treatment, particularly when it correlates with the improvement in SPECT scan.

## Personalized Nutrition

Nutrition plays a crucial role in health as well as disease. Nutraceuticals are dietary supplements such as vitamins, minerals and antioxidants, which can be used for prevention and treatment of diseases. With advances in molecular biology, there is a shift in focus from epidemiology and biochemistry to an understanding of how nutrients act at molecular level. Advances in genomics have led to recognition of the importance of genes in human nutrition. Genetic predisposition is an important factor in mortality linked to diet such as cardiovascular disease. Whereas traditional nutrition research has dealt with providing nutrients to nourish populations, it nowadays focuses on improving health of individuals through diet. Modern nutritional research is aiming at health promotion and disease prevention and on performance improvement.

Technologies such as high-density microarrays enable the simultaneous study of the whole transcriptome relevant to nutrition. Advances in proteomic and metabolomic technologies will also enable the analysis of the whole system at proteomic and metabolomic levels as well. The role of genomics in nutrition is already recognized. The role of metabolomics in nutrition was described in Chap. 7.

## *Nutrigenomics*

The term "nutrigenomics" or nutritional genomics implies the study of effects of nutrition at the genome level. This approach analyzes how a complex trait is produced by the interaction of a person's genes and the environments including nutrition. It also encompasses proteomics as well as metabolomics. A closely related term "nutrigenetics" examines the effect of genetic variation on the interaction between nutrition and disease. Nutrients can alter molecular processes such as DNA structure, gene expression, and metabolism, and these in turn may alter disease initiation, development, or progression. Individual genetic variation can influence how nutrients are assimilated, metabolized, stored, and excreted by the body. A major methodological challenge and first prerequisite of nutrigenomics is integrating genomics, transcriptomics, proteomics and metabonomics to define a "healthy" phenotype. The use of new and innovative technologies, such as microarrays, RNA interference (RNAi), and nanobiotechnologies, will provide needed insights into molecular targets for specific bioactive food components and how they harmonize to influence individual phenotypes. It is important to recognize that an individual's response to dietary intervention will depend on his or her genetic background and that this information may be used to promote human health and disease prevention (Trujillo et al. 2006). The long-term deliverable of nutrigenomics is personalized nutrition for maintenance of individual health and prevention of disease.

Nestle Research Center (Lausanne, Switzerland), a part of the world's largest nutrition company, is conducting research in nutrigenomics. There is a Center of Excellence for Nutritional Genomics at University of California at Davis. Research and postgraduate training in nutrigenomics is being conducted at the Center for Human NutriGenomics in the Netherlands (http://www.nutrigenomics.nl). For nutrigenomics to realize its potential, large ethnically diverse databases of genomic profiles need to be established.

There is increasing popularity of nutrigenomics as both a field of research and as a commercial vehicle for the nutrition and diet foods industries. Commercial kit providers may be misleading consumers by linking diet and DNA via unproven means. Some claims have been made that certain food interacts with genes to increase the risk of certain diseases. The ESRC Center for Genomics in Society at the University of Exeter in UK (http://www.genomicsnetwork.ac.uk/egenis/) funded by the Wellcome Trust, plans to "challenge" corporate and government assertions "that we should alter our diets in accordance with our genetic makeup.

A central theme of the research will be to consider whether or not there should be regulations governing the nutrigenomics and what such regulations should look like. ESRC also plans to investigate what the public is being told by commercial kit providers.

### Genomics of Vitamin D and Calcium Supplementation

Inter-individual response differences to vitamin D and Ca supplementation may be under genetic control through vitamin D and estrogen receptor genes, which may influence their absorption and/or metabolism. Metabolomic studies on blood and urine from subjects supplemented with Ca and vitamin D reveal different metabolic profiles that segregate with genotype. Genotyping was performed for estrogen receptor 1 gene (ESR1) and vitamin D receptor gene (VDR) in postmenopausal women some of whom were classified as low bone density as determined by a heel ultrasound scan and others had normal bone density acting as controls (Elnenaei et al. 2011). Those with low bone density (LBD) were supplemented with oral Ca and vitamin D and were classified according to whether they were 'responders' or 'non-responders' according to biochemical results before and after therapy compared to controls receiving no supplementation. Metabolomic studies on serum and urine were done for the three groups at 0 and 3 months of therapy using NMR spectroscopy with pattern recognition. The non-responder group showed a higher frequency of polymorphisms in the ESR1 (codons 10 and 325) and VDR (Bsm1 and Taq1), compared with to the responders. The wild-type genotype for Fok1 was more frequent in those with LBD (70 %) compared with the control group (10 %). Distinctive patterns of metabolites were displayed by NMR studies at baseline and 3 months of posttreatment, segregating responders from nonresponders and controls. Identification of potential non-responders to vitamin D and Ca, before therapy, based on a genomic and/or metabolomic profile would enable targeted selection of optimal therapy on an individual basis.

### Nutrigenomics and Functional Foods

Functional foods are nutrients that benefit human health beyond the effect of fulfilling essential physiological needs. Many claims have been made for the benefits of functional foods but there are no consistent and proven results, partly because human responses are variable. Polymorphisms in genes for the absorption, circulation, or metabolism of essential nutrients, such as n-3 polyunsaturated fatty acids, would affect the efficacy of that nutrient. However, functional foods often incorporate bioactive compounds, such as epigallocatechin-3-gallate, without considering the interaction with genetic polymorphisms. There are individuals whose genotype precludes their deriving significant benefit from an increased intake of such foods. Although large-scale, whole-genome association studies are providing

an understanding of the genetic basis of health and chronic disease, there is lack of consideration of the interaction with environmental exposure such as to diet. There is need for further studies on gene-diet interactions that may enable rational selection of functional foods leading to optimal health or reduced risk of chronic disease (Ferguson 2009). This information would be useful for personalized nutritional counselling.

### Nutrigenetics and Personalized Medicine

Interindividual genetic variation is an important determinant of differences in nutrition requirements. A common genetic polymorphism results from a C→T substitution in the gene encoding methylenetetrahydrofolate reductase (MTHFR), leads to metabolic changes that modify risk for chronic disease and neural tube defects when accompanied by folate deficiency. The modulation of these metabolic abnormalities by increasing folate intake suggests that folate requirements may be different in affected individuals (T/T) relative to normal (C/C) or heterozygous (C/T) individuals.

Study of nutrigenetics may provide a way of determining responses to bioactive food components and to evaluate these components as biomarkers for predicting risk and tumor behavior. In 2011, the National Cancer Institute (NCI) of USA was asking interested researchers and other parties to provide suggestions about how to design dietary intervention trials to study nutrigenetic approaches to preventing, treating, or making predictions about cancer. There is an increasing amount of evidence that points to diet as a modifier of cancer risk and tumor behavior, but there are many inconsistencies in the literature. Genetic variants involved in food absorption, metabolism, and excretion could serve as biomarkers for predicting which individuals will respond best to dietary modifications for preventing or treating cancer. Such genetic interactions could also potentially be used to predict which individuals may be at risk when consuming suboptimal diets at low, exaggerated, or excessive intakes. NCI is interested in advanced studies in these areas, including research into diet-sensitive genetic modifications that could be used as cancer intervention studies, bioactive food components that warrant further study, specific processes related to genes with molecular targets that are diet sensitive, and other critical issues that are limiting this area of investigation.

### Nutrigenomics and Personalized Medicine

SNPs are powerful tools for investigating the role of nutrition in human disease and may help to define optimized diets in individuals. In future it may lead to adjustment of dietary recommendations on the basis of genotype – personalized diet.

Nutrigenomics holds the promise to revolutionize both clinical and public health nutrition practice by better targeted nutritional interventions (including micronutrient fortification) and facilitate individualized medical nutrition therapy for disease

management to maximize benefit and minimize adverse outcomes within genetically diverse human populations (Stover and Caudill 2008). Research in nutrigenomics may discover pathways that are potentially useful for discovering new therapeutics, particularly for diseases related to metabolism and nutrition such as the following:

- Diabetes
- Obesity
- Cardiovascular diseases
- Some neurological disorders
- Disorders of aging
- Cancer

## *Nutrition and Proteomics*

Scientists at the Nestlé Research Centre (Lausanne, Switzerland) are employing proteomics to address questions of nutrition and health. Nestlé believes that foods and drinks affect individual consumers differently. A food may be well-tolerated by one individual cause but cause violent gastric discomfort in another. Food preference may be related to biomarkers. It is worthwhile to investigate genes that are activated by specific foods for enhancing health and wellness. Certain individuals are more predisposed than others to conditions like obesity or diabetes. If protein markers that indicate such predisposition can be identified before disease symptoms arise, dietary approaches could be devised for health promotion and disease prevention. Nestlé is now including genomics and proteomics approaches into consumer research to impart the health and wellness dimension and to more accurately address individual differences in terms of response to diet and food preference. The long-term deliverable of "Omics" driven food research is personalized nutrition. Proteomics adapted and applied to the context of nutrition and health has the potential to deliver biomarkers for health and comfort, reveal early indicators of disease disposition, assist in differentiating dietary responders from non-responders, and, last but not least, discover bioactive, beneficial food components (Kussmann and Affolter 2006).

## *Personalized Diet Prescription*

Individual response to diet varies; two persons can eat exactly the same diet and respond very differently to it. Genetic variations can explain why some individuals can maintain their weight on a certain diet whereas others gain weight. Diet chemicals can bind to receptors and regulate genes. For example, genestein, a chemical in soy, attaches to estrogen receptors and starts regulating genes. Individual variations in estrogen receptors lead to different reactions to genestein. Genotype and diet interactions contribute to the incidence and severity of obesity, atherosclerosis, certain cancers, asthma, and other chronic conditions. The overall integration of data

and information from the building blocks of metabolism-based nutrient-gene interaction can lead to future individualized dietary recommendations to diminish cancer risk (Go et al. 2005).

Application of genomics in nutrition is important in nutritional management of obesity and special diets for certain diseases such as hypertension (low salt diet). At least one company, NutraGenomics Inc, is using a systems biology approach involving nutrition and the latest molecular and genomic technologies. NutraGenomics will identify diet-regulated genes and nutritional interventions that will allow individuals to better manage their health and well-being. It is anticipated that such a service might be integrated in diets prescribed by physicians as personalized medicines approach is established in medical practice by the end of the first decade of the twenty-first century. Individualized diet prescriptions, based on DNA and protein analysis of a blood sample, may be provided.

Personalized nutrition, however, faces more challenges for implementation than personalized medicine. Whereas drugs are designed to interact with specific targets and the knowledge of sequence of a single gene may be enough to determine whether a person will respond to a drug. In contrast, the diet contains thousands of nutrients, vitamins, and other compounds that can have subtle but significant effects on numerous targets and genes. It may take decades to test the effects of changes in diet, making it difficult to design and conduct well-controlled clinical trials. Simply knowing the sequence of an individual's genes is not enough; there is also a need for understanding how different genes interact as well as of epigenomics – transient modifications in the genome.

## Personalized Physical Exercise

Physical exercise is generally considered to be important for maintaining health and is an important consideration in preventive medicine. However, individuals differ in their responses to physical exercise.

### *Variations in Response to Aerobic Exercise*

Physiological responses to exercise and likelihood of aerobic benefit varies considerably among individuals. Low maximal oxygen consumption (VO2max) is a strong risk factor for premature mortality and endurance exercise training increases VO2max with a very wide range of effectiveness in humans. A study has used RNA expression profiling to produce a molecular classifier that predicts VO2max training response to predict gains in maximal aerobic capacity following endurance exercise training in humans (Timmons et al. 2010). The study concluded that combining RNA profiling with single-gene DNA marker association analysis yields a strongly validated molecular predictor with meaningful explanatory power. $VO_{2max}$ responses to endurance training can be predicted by measuring a 30-gene RNA expression

signature in muscle prior to training. The general approach taken could accelerate the discovery of genetic biomarkers, sufficiently discrete for diagnostic purposes, for a range of physiological and pharmacological phenotypes in humans.

XRPredict (XRGenomics) is multi-gene DNA test that has been scientifically validated for predicting responses to exercise in human trials and is available to consumers from the company after sending a buccal smear sample. A report interpreting the test results is provided. DNA score test result highlight whether a person is a high, normal or low responder to aerobic exercise. The report might suggest that if one is a "low" responder to endurance exercise, one should concentrate on resistance training or refocus the training program. This test is more reliable with a stronger scientific basis than other exercise-related DNA test kits available in the market. However, the value of this test is limited to VO2max response. The test does not tell, for example, how exercise might affect the individual's blood pressure over the long haul, or whether the insulin sensitivity might change, or if the person would be able to lose weight. The genetic markers related to these health responses to exercise are quite different from those related to VO12 max. The gene profile accounts for at ~23 % of the variation in how people responded to endurance training, leaving the rest 77 % of response to exercise up to the individual.

### *Variations in Exercise-Induced Muscle Hypertrophy and Strength*

Gains in skeletal muscle mass with resistance training are also highly variable between individuals from no change to ~60 % increases in muscle size. There are a number of factors that might affect the hypertrophic response, including nutritional support and genetic variation, and a few individual genetic polymorphisms have been identified that may explain a small degree of variability in the resistance training-induced hypertrophic or strength gain phenotype. Muscle hypertrophy, like the response of muscle to endurance training, is regulated by a complex series of partially redundant signaling molecules, including the mTORC1 complex. Muscle hypertrophy is limited by the availability of muscle satellite cells or variations in their ability to proliferate and integrate in vivo that could involve RNA as well as protein factors. miRNAs may contribute to heterogeneous muscle hypertrophy because they have been shown to influence skeletal muscle satellite cell proliferation and differentiation. Several highly expressed miRNAs are selectivity regulated in subjects that represent the lowest 20 % of responders in a longitudinal resistance training intervention study (Timmons 2011).

## Personalized Surgery

Surgery has been traditionally more personalized than drug therapy. Decision to use surgery and choice of procedure are often tailored to individual patients. Surgery for some conditions, genotype studies may influence the decision for surgery.

An example is weight loss surgery. The magnitude of weight loss-induced high-density lipoprotein cholesterol (HDL-C) changes may depend on genetic factors. Association of SNPs in the gene loci that contribute significantly to plasma HDL-C levels in obese individuals at baseline persist at follow-up 10 years after bariatric surgery even after considerable weight loss due to bariatric surgery (Sarzynski et al. 2011). The authors did not observe any associations with bariatric surgery-induced changes in HDL-C levels. The results show that the genetic variants contributing to overall HDL-C levels in apparently weight-stable individuals have little effect on inter-individual variation in the changes of HDL-C in response to the weight loss induced by bariatric surgery. A better understanding of the SNP-HDL associations in obesity and after weight loss surgery could be used as an aid for improving risk prediction and in determining the best treatment options for obese patients.

Risks/benefits are carefully weighed before embarking on surgical procedures. Even in standard textbook procedures, the surgeon often modifies the approach according to the findings and other anatomical variables that may be encountered.

Algorithms for patient management may contain medical and surgical alternatives, combination of both, or surgery as the only choice after failure of medical treatment. Improved understanding of the molecular basis of disease and refinements in molecular diagnostics have contributed considerably to the decision making process as well as prediction of outcome of surgery. Role of surgery, wherever applicable, is described in the personalized management of various diseases in other chapters. Surgery is most frequently integrated with medical management and diagnostics in case of cancer and neurological disorders.

Response to other non-pharmacological methods may be used to make decision about surgery. Some of these methods can also be personalized and may be combined with surgery. Examples are personalized radiotherapy in management of cancer and personalized hyperbaric oxygen.

Increasing emphasis on personalized medicine with integration of diagnostics and surgery will likely reduce the need for surgery as well as failed surgical procedures and complications of surgery. Surgery of the future is also being refined with integration of new technologies such as robotics and minimization of the invasive and traumatic process inherent in surgery.

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# Chapter 20
# Development of Personalized Medicine

## Introduction

In conventional medical practice, the physicians rely on their personal experience in treating patients. In spite of advances in basic medical sciences and introduction of new technologies, the physicians continue to rely on their judgment and sometimes intuition because practice of medicine is an art as well as a science.

Physicians of the last generation had a limited access to information. With advances in molecular biology and its impact on medicine, tremendous amount of new basic information has been generated particularly in genomics and gene expression. Digitalization of information has made it accessible. The problem now is a flood of information, which requires strategies to sort out the relevant from the irrelevant. Information on large number of studies with stratification of large number of patients will have to be analyzed to make decisions about treatment of an individual. The massive amount of publications need to be sorted out and analyzed for their relevance to individualized treatment.

## Players in the Development of Personalized Medicine

Development of personalized medicine is a multidisciplinary undertaking and will need teamwork by many players. Pharmaceutical and biotechnology companies have taken a leading role in this venture in keeping with their future as healthcare enterprises rather than mere developers of technologies and manufacturers of medicines. The practicing physicians will play a vital role in implementing personalized medicine. Various players in the development of personalized medicine are listed in Table 20.1. The Personalized Medicine Coalition contains many of these players.

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**Table 20.1** Players in the development of personalized medicine

| Player | Recommended role |
|---|---|
| Academic medicine | Improved undergraduate education in molecular medicine |
| | Development of a new system of disease classification based on emerging molecular information |
| | Research in molecular medicine |
| Bioinformatic sector | Development of effective clinical decision support tools for integration into electronic health records |
| | Data collection in targeted personalized medicine areas |
| Biotechnology industry | Research and development in molecular medicine technologies |
| Clinical laboratories | Providing companion diagnostic tests for therapeutic agents |
| Governments | Generation of transparent privacy laws |
| | Financial support for research in personalized medicine |
| Health insurance carriers | Providing coverage for personalized medicine |
| | Supporting cost-effectiveness studies of personalized healthcare |
| Organizations for promotion of personalized medicine | Various national and international associations of companies, academic institutions, professionals and lay persons |
| Patients | Increasing participation in health and well-being initiatives |
| | Providing data for research purposes, including social networks |
| Pharmaceutical companies | Development of personalized medicines |
| | Collaboration with molecular diagnostic companies |
| Physicians in private practice | Continuing education in personalized medicine |
| | Introduction of tested personalized medicine approaches in practice |
| Regulatory agencies | Regulations to safeguard patient safety |
| | Expediting decisions for approval of new personalized therapies |



## *Personalized Medicine Coalition*

Personalized Medicine Coalition (http://www.personalizedmedicinecoalition.org/), located in Washington DC, is an independent, non-profit organization of leading pharmaceutical, diagnostic, biotechnology and information technology companies, as well as major academic institutions and governmental agencies. Members of the coalition are shown in Table 20.2.

The Personalized Medicine Coalition (PMC) was formed to fulfill a need for a nationwide, multi-industry policy consensus for personalized medicine. It provides a structure for achieving consensus positions on crucial public policy issues and serves as a forum for debate and education. The strength of the PMC is its multi-disciplinary approach to regulatory, scientific, legal and public policy issues. Its functions are:

- To provide forums for public policy discussions
  - Personalized medicine: science, policy, and economics
  - Public attitudes toward genetics
  - Personalized medicine and cancer

**Table 20.2** Members of the personalized medicine coalition

| | |
|---|---|
| **Industry** | Procognia |
| Abbott Laboratories | Qiagen |
| Affymetrix | Siemens |
| Amgen | Theranos |
| AstraZeneca | **Industry & Consumer Policy** |
| Cogenics/Clinical Data | American Clinical Labs Association |
| DNA PrintGenomics | Biotechnology Industry Organization |
| Exagen Diagnostics | Genetic Alliance |
| Feinstein Kean Healthcare | PEW Genetics & Public Policy Center |
| Gene Logic | Pharmaceutical Research & Manufacturers of America |
| Genentech | |
| Genomas | **Agency Partners** |
| Genomic Health | Centers for Disease Control and Prevention |
| Genzyme Inc | Center for Medicare and Medicaid Services |
| IBM Life Sciences Inc | National Cancer Institute |
| Millennium Pharmaceuticals | National Human Genome Research Institute |
| Monogram Biosciences | FDA |
| Pathway Diagnostics | **Academia** |
| Perlegen Sciences | Duke Univeristy (Durham, NC) |
| Pfizer | George Washington University (Washington, DC) |
| Princeton Group | Harvard Medical School-Partners (Boston, MA) Healthcare Center for Genetics and Genomics |

  - Personalized medicine and psychiatry
  - Public attitudes and trends toward genomics
  - Personalized medicine and reimbursement
  - 'Race' and medicine in the genomics era
- To develop and conduct educational programs for stakeholder audiences
  - Serve as clearing house for information
  - Inform and educate the public and the media
- To facilitate dialogue between industry, government, patients, physicians and other stakeholders leading to consensus solutions

## Role of Pharmaceutical Industry

The pharmaceutical industry has taken the major initiative in the development of personalized medicine. Ten of the major pharma companies are profiled in part II of the report. This interest parallels the applications of knowledge gained from sequencing the genome in drug development and molecular diagnostics. Use of pharmacogenetics and pharmacogenomics in clinical trials sponsored by the pharmaceutical industry is increasing as described in earlier chapters of this report.

In recent history, the pharmaceutical industry has played the major role in developing most of the innovations in therapy. Major pharmaceutical companies have the resources to do so. Eventually for clinical applications, the collaborations involve academic healthcare centers that have the patients. The major incentive for the pharmaceutical industry to participate in the development of personalized medicine is the increasing interest and technologies available for developing such medicines. In future, we will see more competition among the companies in this area as those who do not remain on the forefront will be at a considerable disadvantage in the future healthcare market. Companies such as Hoffmann-La Roche are in a good position to develop such innovative healthcare systems as they have the largest molecular diagnostic facility and already have products where diagnostics and therapeutics are packaged together. Individual technologies and data for the development of personalized medicine stem mostly from biotechnology companies. Principles of personalized medicine play an important role at all stages of the drug development process.

## *Personalized Drug Discovery*

To start with use of established drugs is being personalized. The next step is discovery of personalized drugs. Assays of drug action typically evaluate biochemical activity; however, accurately matching therapeutic efficacy with biochemical activity is a challenge. High-content cellular assays seek to bridge this gap by capturing broad information about the cellular physiology of drug action. The detailed information contained in genomic expression data is sufficient to match the physiological effect of a novel drug at the cellular level with its clinical relevance. This capacity to identify therapeutic efficacy on the basis of gene expression signatures in vitro has potential utility in drug discovery and drug target validation relevant to personalized medicine.

The availability of genomic samples in large phase IV trials provides a valuable resource for further understanding the molecular basis of disease heterogeneity, providing data that feeds back into the drug discovery process in target identification and validation for the next generation of improved medicines.

## *Personalized Approach to Clinical Trials*

It is well recognized that average treatment effects estimated by systematic reviews of clinical trials do not really apply to an individual patient, and might differ in patient subgroups. This can lead to treatment of patients for whom the treatment is not effective, and may be harmful. Positive clinical trial results may mask a lack of meaningful benefit for those at lower risks of illness, e.g. trials involving statins, anticoagulant therapies, and some common surgical procedures (Kent et al. 2010). The authors emphasized that the problem of trials masking the "heterogeneity of

treatment effects" can result in guidelines that promote overtreatment as well as undertreatment, and recommended estimation of treatment effects after stratifying trial participants according to baseline risk. Better stratification of persons by disease stage, or baseline risk of relevant outcomes, is more likely to identify those who will benefit and those who will be harmed by an intervention, leading to the development of appropriate diagnostic and treatment thresholds, ultimately reducing overdiagnosis as well as overtreatment (Moynihan et al. 2014). This approach is in line with personalized medicine. Pharmacogenomic approach to clinical trials is discussed in Chapter and other measures to improve clinical trials are discussed in the following sections.

**Use of Bayesian Approach in Biomarker-Based Clinical Trials**

Innovative clinical trial designs are needed to address the difficulties and issues in the development and validation of biomarker-based personalized therapies. A new clinical trial design that captures the strengths of the frequentist and Bayesian approaches has been proposed to address some of these issues (Lai et al. 2012). There are advantages of using likelihood inference and interim analysis to meet the challenges in the sample size needed and in the constantly evolving biomarker landscape and genomic and proteomic technologies.

The statistical method used nearly exclusively to design and monitor clinical trials today, a method called frequentist or Neyman-Pearson (for the statisticians who advocated its use), is so narrowly focused and rigorous in its requirements that it limits innovation and learning. A solution is to adopt a system called the Bayesian method, a statistical approach more in line with how science works. The main difference between the Bayesian approach and the frequentist approach to clinical trials has to do with how each method deals with uncertainty, an inescapable component of any clinical trial. Unlike frequentist methods, Bayesian methods assign anything unknown a probability using information from previous experiments. In other words, Bayesian methods make use of the results of previous experiments, whereas frequentist approaches assume we have no prior results. This approach is being put to the test at M. D. Anderson Cancer Center (Houston, TX), where >100 cancer-related phase I and II clinical trials are being planned or carried out using the Bayesian approach. The Bayesian approach is better for doctors, patients who participate in clinical trials and for patients who are waiting for new treatments to become available. Physicians want to be able to design trials to look at multiple potential treatment combinations and use biomarkers to determine who is responding to what medication. They would like to treat that patient optimally depending on the patient's disease characteristics. If interim results indicate that patients with a certain genetic makeup respond better to a specific treatment, it is possible to recruit more of those patients to that arm of the study without compromising the overall conclusions. Use of the Bayesian approach may make it possible to reduce the number of patients required for a trial by as much as 30 %, thereby reducing the risk to patients and the cost and time required to develop therapeutic strategies.

Using a Bayesian approach, contrary to the standard approach, the trial design exploits the results as the trial is ongoing and adapts based on these interim results. In order to have the personalized medicine, it will be necessary to be more flexible in how we evaluate potential new treatments. Moreover, it is possible to reduce the exposure of patients in trials to ineffective therapy using the Bayesian approach. Whether the Bayesian approach will gain acceptance in clinical trials depends a lot on its acceptance by the FDA in determining safety and efficacy of new treatments. The FDA has already approved the Bristol-Myers Squibb drug Pravigard Pac for prevention of secondary cardiac events based on data evaluated using the Bayesian approach.

### Individualizing Risks and Benefits in Clinical Trials

COX-2 inhibitors such as rofecoxib, celecoxib, and valdecoxib confer a small, but absolute, risk of heart attack and stroke. The size of this risk is likely to be conditioned by the underlying risk in a given patient of thrombosis and heart disease; the dose and duration of action of a drug; and the duration of dosing and concurrent therapies, such as low-dose aspirin. Among the questions that remain to be addressed are the following: (a) whether this hazard extends to all or some of the traditional NSAIDs; (b) whether adjuvant therapies, such as low-dose aspirin, will mitigate the hazard and if so, at what cost; (c) whether COX-2 inhibitors result in cardiovascular risk transformation during chronic dosing; and (d) how we might identify individuals most likely to benefit or suffer from such drugs in the future. Lessons are drawn from the experience of the COX-2 inhibitors-particularly the need to develop a more interdisciplinary approach to drug development and monitoring of drug safety and how an emphasis on individualizing benefit and risk can be used to refine the design of clinical trials.

A study that builds on the theme of individualized therapy, has demonstrated marked variation in individual response to COX-2 inhibitors, as measured by plasma drug levels and the degree of COX-2 inhibition within an individual (Fries et al. 2006). The researchers found a marked degree of variability in individuals dosed with either rofecoxib or celecoxib, even when they studied apparently healthy, relatively young individuals in a carefully controlled environment. This rigorous study suggests ~30 % of variability found in patients is attributable to differences between individuals, suggesting the contribution of genetics to a variety of biomarkers of drug response. Exploitation of variability in response can lead to tests which identify patients most likely to benefit or suffer from drugs. This study provides a starting point for the development of diagnostics that will enable conservation of benefit while managing the risk of COX-2 inhibitors.

### Clinical Trials of Therapeutics and Companion Diagnostics

Clinical trial designs and adaptive analysis plans for the prospective design of pivotal trials of new therapeutics and companion diagnostics require a careful analysis strategy (Simon 2008). The target populations for analysis should be prospectively

specified based on the companion diagnostic. Clear separation is generally required of the data used for developing the diagnostic test, including their threshold of positivity, from the data used for evaluating treatment effectiveness in subsets determined by the test. Adaptive analysis can be used to provide flexibility to the analysis but the use of such methods requires careful planning and prospective definition in order to assure that the pivotal trial adequately limits the chance of erroneous conclusions.

## *Role of Drug Delivery in Personalized Medicine*

Along with other technologies, refinements in drug delivery will play an important role in the development of personalized medicine. One well known example is glucose sensors regulating the release of insulin in diabetic patients. Gene therapy, as a sophisticated drug delivery method, can be regulated according to the needs of individual patients. ChipRx Inc is developing a true "responsive therapeutic device" in which biosensors, electronic feedback and drug/countermeasure release are fully integrated.

## *Repositioning of Drugs for Personalized Medicine*

Repositioning or repurposing of a drug means its use for an indication other than originally intended. The pharmaceutical industry is exploring this approach because of high failure rate of drugs in development and paucity of new drugs in pipelines. The advantage of repositioning the drug is shortening of development time as the drug has already passed toxicity testing and safety assessment and needs only late stage clinical trials for the new indication. For an approved drug, development of an additional indication may be initiated by feedback from clinicians' off-label use of the drug. With increasing knowledge of genomic basis of diseases, repositioning may be useful for matching the right drug to the right patient.

There are numerous examples of drugs that have been repositioned. Gabapentin and pregabalin, originally developed as antiepileptic drugs, are used more often for neuropathic pain. Sildenafil (Viagra) was initially developed and studied for use in hypertension and angina pectoris, for which it was not adequately effective. Observation of penile erection as side effect led to its development for erectile dysfunction. Other indications are also being explored.

Bisphosphonates are a commonly prescribed therapy for osteoporosis and skeletal metastases. The drugs have also been associated with reduced tumor burden in some patients, but the mechanism is unknown. A study has provided evidence that bisphosphonates inhibit EGFR (HER) receptor tyrosine kinase, including the commonly mutated forms that drive NSCLC, as well as a resistance mutation (Stachnik et al. 2014). This new mechanism lays the basis for the repositioning of bisphosphonates for the prevention and therapy of HER family-driven cancers.

### *Production and Distribution of Personalized Medicines*

With adoption of personalized approaches, there will be changes in production and distribution of pharmaceutical products. Possible scenarios are:

- The drug may be manufactured as previously but the amount manufactured may be less due to restricted use to a certain genotype.
- The drug may be split into batches with slight variations of the basic structure in each. This may require modifications of the manufacturing process.
- If a drug is linked to a diagnostic, both may be packed together but it will not affect the basic manufacturing process.
- In case of biologicals that may be customized according to the group or even an individual, the procedures have to be flexible based on the input from clinical use.

It is beyond the scope of this report to go into the manufacturing methods, which will obviously need to be modified for personalized medicines. Scientists involved in this area will have to become familiar with personalized medicine. Automated systems may be developed in future that may translate biological factors into manufacturing modifications required for individuals. An extreme scenario is filling of a prescription for a personalized drug finalized by a pharmacist at the pharmacy terminal based on a manufacturing process starting at the pharmaceutical company.

The economic aspects of such a modification will need to be worked out in detail for each product. According to the general statements made about the commercial aspects, personalized medicine may cost more to manufacture but can be priced higher than conventional medicines. Currently, it appears unlikely that a major biopharmaceutical company will provide a biological therapy that is custom made from a patient's tissues, e.g. a tumor vaccine based on the patient's cancer. Such a service is currently provided by smaller biotechnology companies.

The FDA is beginning to address these issues with a new initiative using a "risk-based approach" that employs the principles of Process Analytical Technology (PAT). PAT involves the design of in-line, on-line or at-line sensors that operate at critical points in a pharmaceutical manufacturing operation. These sensors will markedly reduce the cost of producing pharmaceutical products by allowing manufacturing activities to become decentralized. This will, in turn, allow for the manufacture of "personalized medicines" and broaden the number of therapeutic agents and drug delivery systems available for treating human disease by reducing stability and scale-up concerns that might ordinarily prevent life-saving therapies from becoming products. The University of Kentucky proposes to develop a center that would contribute to sensor research as well as address critical unmet needs of the FDA initiative: facilities for integrating sensor technology with lean manufacturing and visualization/virtual environments. The Center will be designed to complement existing research centers, federal funding agencies, and industrial initiatives focused on modern manufacturing processes for the pharmaceutical industry.

## Role of Biotechnology Companies

Most of the biotechnology companies profiled in part II of this report are involved in pharmacogenomics, pharmacogenetics, pharmacoproteomics and molecular diagnostics. Smaller biotechnology companies that may invent or develop technologies for advancing personalized medicine depend on collaborations with major pharmaceutical companies. Some of these companies are already on the way to become pharmaceutical companies. Apart from academic collaborations, many of these companies have alliances with other biotechnology companies as well as with pharmaceutical companies. Some of the companies are now dedicated as personalized medicine companies whereas others continue to categorize themselves in the basic technologies for personalized medicine. All of them play a role in the development of personalized medicine, which is not the exclusive domain of any one company.

## Role of Life Sciences Industries

In 2006, life science industry leaders formed a BioIT Alliance (http://bioitalliance.org/). The founding members including Accelrys Software, Affymetrix, Amylin Pharmaceuticals, Life Technologies and The Scripps Research Institute, which share a common goal of creating a stronger link between technology and science. The alliance unites the pharmaceutical, biotechnology, hardware and software industries to explore new ways to share complex biomedical data and collaborate among multidisciplinary teams to ultimately speed the pace of drug discovery and development. By bringing together people from innovative life sciences organizations that span the biomedical industry, the BioIT Alliance will play an important role in the development of solutions that transform today's data into knowledge and improve the quality of millions of lives. Life science companies have unique technical challenges such as the need for more comprehensive data integration solutions, better technical collaboration and stronger knowledge management capabilities. The BioIT Alliance brings together science and technology leaders to consider innovative ways to address these challenges and use technology to reduce costs, streamline research and market their products more effectively. Founding members of the alliance have already begun to collaborate on solutions that target common technology problems faced by life science companies.

The first of these solutions is the Collaborative Molecular Environment, which will provide a means for data capture, visualization, annotation and archiving using Microsoft® Office, Windows® Presentation Foundation and SharePoint® Technologies. Microsoft is partnering with alliance member company InterKnowlogy LLC on the project, which is being tested by several other alliance members. In addition to making data easier to manage, early efforts of the alliance are focused on making data easier to share. Two member companies working on this are Affymetrix and Life Technologies. The BioIT Alliance will also provide independent software

vendors with industry knowledge that helps them to commercialize informatics solutions more quickly with less risk. Most efforts to unite the life science and information technology industries are focused on developing technology to enable the early-stage drug discovery process. By addressing the technology issues that companies face throughout the development cycle and by working with some of world's top technology providers, the alliance will help the industry move closer to making personalized medicine a reality.

## *Role of the Clinical Laboratories*

The role of the clinical laboratories in pharmacogenomics is established now, as there are several such facilities that provide technologies to improve the efficacy and safety in drugs by using genetic testing to determine patient therapy. Currently, clinical laboratories assist pharmaceutical sponsors in preclinical pharmacogenetic testing. In the future clinical laboratories will participate in genetic test development and validation, high-throughput genotyping of patients in clinical trials, and personalized medicine.

However, when molecular diagnostic technology advances to point-of-care stage, a patient's genotype may be determined on the spot and not sent to a laboratory. Similarly, with merging of diagnostics and therapeutics in integrated healthcare, diagnostic kits may be sold along with the therapeutics and laboratory procedures would be done at the comprehensive healthcare clinics. Clinical laboratories, however, will continue to serve pharmaceutical industry during the drug development stage. The volume of SNP genotyping required for clinical trials would be beyond the capacity of any on-site testing system and would be better delegated to a clinical laboratory. Moreover, the quality control of such testing or regulatory oversight may not be possible unless an approved laboratory conducts these tests. To keep up with the challenges of the future, clinical laboratories will have to get involved in research in pharmacogenomic technologies and participate in the development of tests.

## *Role of Molecular Imaging in Personalized Medicine*

Technologies encompassed within molecular imaging include optical, magnetic resonance imaging (MRI) and nuclear medicine techniques. Positron emission tomography (PET) is the most sensitive and specific technique for imaging molecular pathways in vivo in humans. PET uses positron emitting radionuclides to label molecules, which can then be imaged in vivo. The inherent sensitivity and specificity of PET is the major strength of this technique. Indeed, PET can image molecular interactions and pathways, providing quantitative kinetic information down to subpicomolar levels. Generally, the isotopes used are short-lived. Once the molecule is

labeled, it is injected into the patient. The positrons that are emitted from the isotopes then interact locally with negatively charged electrons and emit what is called annihilating radiation. This radiation is detected by an external ring of detectors. It is the timing and position of the detection that indicates the position of the molecule in time and space. Images can then be constructed tomographically, and regional time activities can be derived. The kinetic data produced provide information about the biological activity of the molecule. Molecular imaging provides in vivo information in contrast to the in vitro diagnostics. Moreover, it provides a direct method for the study of the effect of a drug in the human body. Personalized medicine will involve the integration of in vitro genotyping and in vivo phenotyping techniques.

**Molecular Imaging for Personalized Drug Development in Oncology**

For decades anatomic imaging with CT or MTI has facilitated drug development in medical oncology by providing quantifiable and objective evidence of response to cancer therapy. In recent years metabolic imaging with 18Ffluorodeoxyglucose-PET has added an important component to the oncologist's armamentarium for earlier detection of response that is now widely used and appreciated. These modalities along with ultrasound and optical imaging (bioluminescence, fluorescence, near-infrared imaging, multispectral imaging) have become used increasingly in preclinical studies in animal models to document the effects of genetic alterations on cancer progression or metastases, the detection of minimal residual disease, and response to various therapeutics including radiation, chemotherapy, or biologic agents. The field of molecular imaging offers potential to deliver a variety of probes that can image noninvasively drug targets, drug distribution, cancer gene expression, cell surface receptor or oncoprotein levels, and biomarker predictors of prognosis, therapeutic response, or failure. Some applications are best suited to accelerate preclinical anticancer drug development, whereas other technologies may be directly transferable to the clinic. Efforts are underway to apply noninvasive in vivo imaging to specific preclinical or clinical problems to accelerate progress in the field. By enabling better patient selection and treatment monitoring strategies, molecular imaging will likely reduce the future cost of drug development.

As anticancer strategies become more directed towards a defined molecular target, we need information that is relevant to humans about whether the molecular target is expressed, the selectivity and binding of the compound for that target, and the effects of such an interaction. The following is an example of the use of molecular imaging in drug discovery for cancer.

p53 deficiency is common in almost all human tumors and contributes to an aggressive chemo- or radiotherapy-resistant phenotype, therefore providing a target for drug development. Molecular targeting to restore wild-type p53 activity has been attempted in drug development and has led to the identification of CP-31398, PRIMA1, and the Nutlins. The use of noninvasive bioluminescence imaging has been demonstrated in a high-throughput cell-based screen of small molecules that activate p53 responses and cell death in human tumor cells carrying a mutant p53

(Wang et al. 2006). A number of small molecules were isolated that activate p53 reporter activity, increase expression of p53 target genes such as p21(WAF1) or death receptor 5 (KILLER/DR5) of TNF-related apoptosis-inducing ligand (TRAIL), and induce apoptosis in p53-deficient cells. Some of the compounds activate a p53 response by increasing p73 expression, and knockdown of transactivating isoforms of p73 by siRNA reduces their induction of p53-responsive transcriptional activity. Some compounds do not induce significant p73 expression but induce a high p53-responsive transcriptional activity in the absence of p53. In vivo experiments demonstrate potent antitumor effects of selected compounds. The results establish the feasibility of a cell-based drug screening strategy targeting the p53 transcription factor family of importance in human cancer and provide lead compounds for further development in cancer therapy. These findings emphasize the growing role of imaging technology in aiding researchers in the development of personalized cancer treatments. The therapeutic effects of the small molecule compounds will be explored in different types of cancer and the potential toxicities of these compounds will be evaluated.

Molecular imaging can provide pharmacokinetic (PK) and pharmacodynamic (PD) information. Use of the technique in early clinical trials can:

- Provide information on optimum biological dose and PK/PD relationships
- Identify tumors containing specific molecular targets
- Provide in vivo pharmacodynamic evaluation of compounds.

Further efforts are needed in this area and pharmaceutical industry need to get involved besides the academic investigators and the companies providing the equipment and other materials. The major challenge for drug development is to overcome the lack of specific tracers and ligands available for in vivo imaging. Here, the problem is often not one of specificity for the molecular interaction or pathway, but rather of background owing to non-specific binding in vivo, peripheral metabolism and/or poor penetration across endothelial barriers. In vivo assays of molecular interactions and pathways should be sufficiently cancer-specific to be of use as therapeutic targets. Such probes could provide therapeutically relevant functional measures of disease status and, hence, assays of potential responsiveness. They would also provide endpoints of pharmacodynamic responses. Systems already in place for cancer include the imaging of proliferation and its relevance to anti-proliferative agents, blood flow and its relevance to antiangiogenic agents, and gene expression with relevance to gene therapy. If an in vivo diagnostic is available to monitor the effects of numerous available antiangiogenesis agents on tumors, it can help us to define responder and non-responders.

CellPoint LLC is optimizing its novel diagnostic imaging agent on Philips' SKYLight® gantry-free gamma camera in collaboration with Philips Medical Systems. CellPoint's ethylenedicysteine (EC) drug conjugate technology is a unique delivery system that functions as a chemical bridge linking tissue specific ligands, such as hormones, proteins, peptides, glucose analogues, or pharmaceutical compounds to radioisotopes for cancer diagnosis and treatment. The companies will collaborate on the molecular imaging agent, Tc-99 m-EC-deoxyglucose based on

EC technology. The joint goal is to develop a cost-effective, readily accessible molecular imaging technology that can help more clinics and hospitals accurately diagnose cancer and pre-screen patients for therapy.

Scientists at various European centers have demonstrated the ability to image a selective increase in uptake of intravenously administered Tc-99 m-rh-Annexin V (North American Scientific Inc's Hynic Annexin) within 1 day of chemotherapy in the responding portion of a tumor subsequently confirmed by a partial response to chemotherapy on a CT scan 6 weeks later. There is a good correlation between the degree of uptake of Hynic-Annexin V measured on images of head and neck tumors and the degree of cell death in the tumors demonstrated on microscopic examination following surgical removal of the tumors. While the Company is awaiting clinical follow-up data from patients enrolled in its European Phase II study of early assessment of response to treatment in patients with non-small cell lung cancer, the range of annexin image findings to date show both response and non-response in study subjects. These findings have been correlated with clinical results of treatment. Molecular imaging would provide the possibility of tailoring anticancer drug therapy on a patient-by-patient basis in accordance with their response.

## Molecular Imaging and CNS Drug Development

There are several examples of the usefulness of molecular imaging in CNS drug development. Use of PET in drug development can unravel the disease mechanism, measure the disease progression, demonstrate drug action in vivo and enable the defining of drug-response curves for phase I and phase II studies. This can speed up drug development. The imaging agent PK11195 (GE Healthcare Bioscience) binds to peripheral benzodiazepine sites at microglia (20 % of all non-neuronal cells in the brain) that are activated by injury or disease. Some applications of this technique as well as other imaging techniques in various CNS diseases are:

**Multiple Sclerosis** $^{11}$C-PK11195 can pick up inflammatory changes in both optic nerves in multiple sclerosis patients, which are not shown on ordinary MRI. It fulfils the need for marker as a guide to interferon therapy of these patients.

**Parkinson Disease (PD)** $^{11}$C-PK11195 PET can be used to follow the progression of inflammation in Parkinson disease and its response to various therapies. $^{18}$F-dopa PET can follow the progression of the disease from detection of dopamine deficit in an asymptomatic PD twin to clinical manifestations 5 years later. This method can also be used to test the effect of neuroprotective drugs in PD. Infusion of glial cell-derived neurotrophic factor (GDNF) into the putamen of PD patients demonstrates significant increases in $^{18}$F-dopa uptake following 2 years of GDNF infusion.

**Alzheimer Disease (AD)** $^{11}$C-PK11195 binding correlates with atrophy of left temporal lobe shown on MRI in AD patients and the course can be followed over a long period. It provides a chance to test various drugs and determine their action, e.g., if they have any neuroprotective effect. $^{18}$F-FDDNP, a hydrophobic radiofluorinated derivative of 2-(1-[6-(dimethylamino)-2-naphthyl]ethylidene)malononitrile

(DDNP), binds to synthetic beta-amyloid(1–40) fibrils, neurofibrillary tangles (NFTs) and amyloid plaques in human AD brain specimens.

$^{18}$F-FDDNP, in conjunction with PET, can be used to determine the localization and load of NFTs and Aβ senile plaques in the brains of living AD patients. Greater accumulation and slower clearance is observed in amyloid plaque- and NFT-dense brain areas and correlated with lower memory performance scores. The relative residence time of the probe in brain regions affected by AD is significantly greater in patients with AD than in control subjects. This noninvasive technique for monitoring AP and NFT development is expected to facilitate diagnostic assessment of patients with AD and assist in response-monitoring during experimental treatments.

There is loss of glucose metabolism in AD usually measured by FDG-PET. This can also be measured by $^{11}$C-PIB and the slope values correlate with the findings of FDG dementia index. $^{123}$I-QNB SPECT can demonstrate M1 muscarinic receptor binding in AD. There is increased M1 binding in donepezil responders as compared to non-responders.

**Commercial Development of Molecular Imaging**

Companies developing molecular imaging have a considerable interest in developing personalized medicine. Three major companies – GE, Philips and Siemens – involved in producing imaging equipments such as MRI and PET for healthcare applications, are interested in these developments. Amersham Healthcare, North American Scientific Inc and Schering AG provide imaging agents.

Molecular imaging and diagnostics (MID) is being developed by Philips Medical Systems, a part of Royal Philips Electronics. New imaging, diagnostic and therapeutic techniques arising from MID will cause a paradigm shift in healthcare procedures. Through time, much more emphasis will be placed on diagnosing and treating symptoms – even providing a cure – before secondary symptoms occur. In contrast, with molecular diagnostics, highly sensitive devices will permit the screening of initial symptoms and that will change the scenario for the next 10–20 years, where the family doctor will be able to screen for very early symptoms, or even treat before symptoms occur. Then, if required, the patient will be referred to a hospital or medical center for further diagnosis and staging, using molecular imaging and targeted contrast agents that can interact with processes in a ‘pre-disease’ state. If treatment is required, new pharmaceutical procedures will allow patient-specific drug delivery, resulting in the ‘prevention rather than the cure’ of a (potential) disease. In the more distant future (after 20 years) screening, staging and treatment will, as can be expected, all be performed at the molecular level, and probably by the family doctor. It is also feasible that screening for certain selected symptoms may be performed at home by the individual without professional medical assistance.

GE Medical Systems (Waukesha, Wisconsin, USA) provides non-invasive molecular imaging methods, such as PET, CT or MRI combined with novel imaging agents. GE Medical Systems is collaborating with biotechnology industry to combine genetic and proteomic data with diagnostic imaging for enhancing early

diagnosis and treatment of cancer and other diseases. Such collaboration will combine the strengths of genomics, functional genomics and molecular imaging to place better information in the hands of healthcare professionals to enable them to genetically determine a patient's risk for developing disease long before any symptoms appear without unnecessary exploratory procedures. The University of Texas M. D. Anderson Cancer Center (Houston, Texas) conducts multi-disciplinary research using these combined technologies.

## Role of the US Government and Agencies in Personalized Medicine

US healthcare system is facing a crisis because of high cost and lack of health insurance for a significant percentage of population. Improvement of healthcare is a priority for the US government. Implementation of personalized healthcare will depend on the final plan that will be implemented. Meanwhile research and development relevant to personalized medicine continues in the US.

A bill was introduced in US Congress in 2006 by Senator Obama, who later became President of the US, titled "Genomics and Personalized Medicine Act of 2006" that aimed to advance personalized medicine and pharmacogenomics. It was replaced later by another bill that included a new tax incentive for personalized medicine research. The Genomics and Personalized Medicine Act of 2008 (H.R.6498) adds tax and test credit incentives to lure researchers into the field. The bill was introduced and referred to the House Ways and Means Committee and to the House Energy and Commerce Committee. The core focus of the act is on the following points:

- It would create a Genomics and Personalized Medicine Interagency Working Group that would include the NIH, the FDA, the Centers for Disease Control and Prevention, and other groups outside of the Department of Health and Human Services.
- It also would start a National Biobanking Initiative that would create a database for collecting and integrating genomics data with environmental and clinical health information. It also would use funding to improve training for diagnosis of genetic diseases and disorders, and for treatment and counseling.
- The final part of the bill would implement an oversight matrix for regulating genetic tests and pharmacogenomic tests, and would encourage the development of companion diagnostics by drug sponsors and by device companies.
- An amendment will include tax credit for research expenses incurred in the development of a companion diagnostic test.

The description of the act focuses on genomics and genetic testing and misses the broad contest of personalized medicine as discussed in this report. Although it is an encouraging step, it remains to be seen if it will facilitate the introduction of personalized medicine and add to the advances already made in the industrial sector.

In 2010, Kennedy/Eshoo "Genomics and Personalized Medicine Act of 2010 – HR 5440" was introduced in the US Congress. Compared to previous personalized bills, including that introduced by Barack Obama in 2006, this bill was more emphatic with an aim to stimulate and accelerate the research and development of products used in personalized medicine and to move these diagnostic and treatment modalities from the laboratory into clinical practice. This goal will be accomplished through the establishment of a human biological specimen repository within the NIH to increase our understanding of diseases, our environment and the genome, and though establishing numerous grants to expand and accelerate the creation and use of products used in personalized medicine. Given the slow creation and adoption of products used for personalized medicine in clinical practice, the legislation establishes the Office of Personalized Healthcare (OPH) to facilitate the coordinated movement of genomics and personalized medicine throughout the Federal government and private sector. The legislation also addresses several issues that have arisen with the increased prevalence of genetic testing, including coverage and reimbursement of personalized medicine products, and oversight of genetic tests (including direct-to-consumer marketing). Given the recent issues surrounding direct-to-consumer marketing of some genetic and genomic tests, the legislation would also direct the FDA, FTC and CDC to evaluate these products that circumvent the both the normal regulatory environment and patient-health care provider relationship.

On 20 January 2015, the President of USA announced a research initiative that aims to accelerate progress toward a new era of personalized precision medicine (www.whitehouse.gov/precisionmedicine). The time is right for this initiative and the NIH as well as other partners will work to achieve this vision (Collins and Varmus 2015).

### *Department of Health and Human Services and Personalized Medicine*

In 2008, the Department of Health and Human Services (HHS) released an update of its ongoing efforts in the personalized healthcare arena, and the vision the outgoing US government had for this new medical area in diagnostics, treatment, and research. The full 300-page report, Personalized Health Care: Pioneers, Partnerships, Progress is available on line at: http://www.hhs.gov/myhealthcare/. In a prologue to the report, meant as a note for the next government, it is explained that personalizing healthcare "is not a niche concern. Its promise is central to the future of healthcare." However, a warning put an effective personalized healthcare system in place as "the work of a generation." According to the report, within 10 years "it will be the norm for consumers and practitioners to anticipate that treatments should be individually targeted, with diagnostics and therapies commonly associated as a paired unit" and "within 15 years major clinical data sources can be securely linked in a manner that gives most Americans the option of allowing their own de-identified health information to be employed in the quest for ever-more individualized understanding of health and disease." It is further stated that "within 20 years data and

informatics will have advanced to the point of supporting meaningful individual prediction regarding an individual's life-long health prospects, including specific, proven steps that he or she can take to protect and enhance health."

Although this report is encouraging, the timeline seems to be close to that of the Royal Society of UK, a critical review of which will be presented later in this Chapter. Personalized medicine has made the most advances in the US. It is expected that the US government will move faster in implementing personalized medicine.

Because HHS administers the Medicare program through Centers for Medicare and Medicaid Services (CMS), HHS will be involved in the area of reimbursement for personalized medicine services within this program. With cost-cutting in the current financial crisis, it is not certain if any expensive innovations will be covered under Medicaid. HHS will also play a role in the regulation of DCT for genetic disorders which is covered under role of FDA.

Across the US, clinicians and patients confront important health care decisions without adequate information. There is a need for answers to the questions:

- What is the best pain management regimen for disabling arthritis in an elderly African-American woman with heart disease?
- What care coordination approach is most effective at preventing hospital readmissions of neurologically impaired children with special health care needs,?
- What treatments are most beneficial for patients with depression who have other medical illnesses?
- Can physicians tailor therapy to specific groups of patients using their history or special diagnostic tests?
- What interventions work best to prevent obesity or tobacco use?

Unfortunately, the answer to these types of comparative, patient-centered questions in health care is often, "We don't really know." Thousands of health care decisions are made daily; patient-centered comparative effectiveness research focuses on filling gaps in evidence needed by clinicians and patients to make informed decisions. Physicians and other clinicians see patients every day with common ailments, and they sometimes are unsure of the best treatment because limited or no evidence comparing treatment options for the condition exists. As a result, patients seen by different clinicians may get different treatments and unknowingly be receiving less effective care. Patients and their caregivers search in vain on the Internet or elsewhere for evidence to help guide their decisions. They often fail to find this information either because it does not exist or because it has never been collected and synthesized to inform patients and/or their caregivers in patient-friendly language. When they do find information, it may be informed by marketing objectives, not the best evidence.

## *Agency for Healthcare Research and Quality*

The American Recovery and Reinvestment Act of 2009 provided $1.1 billion for comparative effectiveness research (CER), including $300 million to the Agency for Healthcare Research and Quality (AHRQ); $400 million for the

NIH; and $400 million for the Office of the Secretary of Health and Human Services. A report by the Federal Coordinating Council for Comparative Effectiveness Research, which was created by the ARRA, proposed that comparative effectiveness should complement the trend in medicine to develop personalized medicine, and should use the ability to investigate drug and dosage effects at the sub-group level in ways that are difficult in randomized trials.

AHRQ provided $100 million in stimulus funding in 2010 to support studies of the effectiveness of treatments and diagnostics, including personalized healthcare approaches, such as pharmacogenetics, clinical diagnostics, and bio-imaging studies. The projects entailed a range of approaches, including prospective studies that explore the outcomes of pharmacogenetic testing in guiding selection of therapeutic interventions, evaluation of new imaging technologies to diagnose or monitor treatments, and prospective and longitudinal cohort studies of effectiveness and comparative effectiveness of diagnostics, devices, and drugs. As a unit of the DHHS dedicated to advancing excellence in health care, AHRQ has the following two programs dedicated wholly or in part to personalized medicine.

*Evidence-Based Practice Centers (EPCs)* These centers review all relevant scientific literature on clinical, behavioral, and organization and financing topics to produce evidence reports and technology assessments. These reports are used for informing and developing coverage decisions, quality measures, educational materials and tools, guidelines, and research agendas. EPCs also conduct research on methodology of systematic reviews. AHRQ awards 5-year contracts to institutions in the US and Canada to serve as EPCs.

*Centers for Education and Research on Therapeutics (CERTs)* These are part of a national initiative to conduct research and provide education that advances the optimal use of therapeutics (drugs, medical devices, and biological products). The program consists of 14 research centers and a coordinating center and is funded and run as a cooperative agreement by AHRQ in consultation with the FDA.

## *Comparative Effectiveness Research*

Due to numerous advances in biomedical science, clinicians and patients often have a plethora of choices when making decisions about diagnosis, treatment, and prevention, but it is frequently unclear which therapeutic choice works best for whom, when, and in what circumstances. Comparative effectiveness research (CER) is the conduct and synthesis of research comparing the benefits and harms of different interventions and strategies to prevent, diagnose, treat and monitor health conditions in "real world" settings. The purpose of this research is to improve health outcomes by developing and disseminating evidence-based information to patients, clinicians, and other decision-makers, responding to their expressed needs, about which interventions are most effective for which patients under specific circumstances. To provide this information, comparative effectiveness research must assess

a comprehensive array of health-related outcomes for diverse patient populations and sub-groups. Defined interventions compared may include medications, procedures, medical and assistive devices and technologies, diagnostic testing, behavioral change, and delivery system strategies. This research necessitates the development, expansion, and use of a variety of data sources and methods to assess comparative effectiveness and actively disseminate the results.

The purpose of CER, supported by the US Government is to provide information that helps clinicians and patients choose which option best fits an individual patient's needs and preferences. It also can inform the health choices of those Americans who cannot or choose not to access the health care system. Clinicians and patients need to know not only that a treatment works on average but also which interventions work best for specific types of patients (e.g. the elderly, racial and ethnic minorities). Policy makers and public health professionals need to know what approaches work to address the prevention needs of those Americans who do not access health care. This information is essential to translating new discoveries into better health outcomes for Americans, accelerating the application of beneficial innovations, and delivering the right treatment to the right patient at the right time.

Examples of successful CER include summaries of evidence from the Agency for Healthcare Research and Quality (AHRQ) on numerous conditions, such as prostate cancer and osteoporosis, as well as the NIH diabetes prevention trial, which demonstrated that lifestyle change was superior to metformin and placebo in preventing onset of type 2 diabetes. Additionally, the Veterans Affairs (VA) COURAGE trial demonstrated that patients treated with optimal medical therapy alone did just as well as patients who received percutaneous coronary intervention plus medical therapy in preventing heart attack and death. These exemplars show the power of CER to inform patient and clinician decisions and improve health outcomes.

Patients increasingly and appropriately want to take responsibility for their care. Therefore healthcare providers have a responsibility to provide comparative information to enable informed decision-making. This patient-centered, pragmatic, "real world" research is a fundamental requirement for improving care for all Americans. Comparative effectiveness differs from efficacy research because it is ultimately applicable to real-world needs and decisions faced by patients, clinicians, and other decision makers. In efficacy research, such as a drug trial for the FDA approval, the question is typically whether the treatment is efficacious under ideal, rather than real-world, settings. The results of such studies are therefore not necessarily generalizable to any given patient or situation. But what patients and clinicians often need to know in practice is which treatment is the best choice for a particular patient. In this way, comparative effectiveness is much more patient-centered. Comparative effectiveness has even been called patient-centered health research or patient-centered outcomes research to illustrate its focus on patient needs.

The American Recovery and Reinvestment Act (ARRA) provided $1.1 billion for comparative effectiveness research. The Act allocated $400 million to the Office of the Secretary HHS, $400 million to the NIH, and $300 million to the HHS Agency for Healthcare Research and Quality. It also established the Federal Coordinating Council for Comparative Effectiveness Research (the Council) to foster optimum

coordination of CER conducted or supported by Federal departments and agencies. Furthermore, the legislation indicated that "the Council shall submit to the President and the Congress a report containing information describing Federal activities on CER and recommendations for such research conducted or supported from funds made available for allotment by the Secretary HHS for CER. The Institute of Medicine considers the following as priority areas for CER studies:

- Comparison of the effectiveness of patient decision support tools on informing diagnostic and treatment decisions.
- Comparison of adding information about new biomarkers (including genetic information) with standard care in motivating behavior change and improving clinical outcomes.
- Diagnostic imaging performed by non-radiologists versus radiologists
- Alternative clinical management strategies for hepatitis C, including alternative duration of therapy for patients based on viral genomic profile and patient risk factors.

## *Role of the US Government Agencies in Personalized Medicine*

Various US Government agencies involved in personalized medicine are: FDA (see under regulatory agencies), NIH, CDC, National Institute of General Medical Sciences, National Institute of Standards and Technology, Centers for Medicare and Medicaid Services, and AHRQ (see preceding section). The work of these agencies is not coordinated.

### Evaluation of Genetic Tests and Genomic Applications

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) group's recommendations are part of a pilot project by the National Office of Public Health Genomics at the US Centers for Disease Control and Prevention. The project aims to evaluate genetic tests and other genomic applications currently in transition from research to clinical use. EGAPP released three new sets of recommendations about gene expression profiling in breast cancer, genetic testing for Lynch syndrome in colorectal cancer patients, and testing for UGT1A1 in colorectal cancer patients treated with irinotecan. These evaluations are in currently in draft form. Of the three recommendations, the one investigating gene expression profiling in breast cancer is the furthest along. There is limited evidence of analytic validity, limited evidence of clinical validity but no direct evidence, i.e. controlled trials testing clinical outcomes or clinical utility. There are mixed estimates of cost-effectiveness. In spite of these concerns, there is a positive balance with potential benefits versus potential harms.

Earlier EGAPP reports evaluated cytochrome P450 testing with AmpliChip (Roche) or other tests to guide physicians treating patients with depression who are taking SSRIs (EGAPP Recommendation Statement 2007). There was insufficient evidence to support a recommendation for or against use of CYP450 testing in

adults beginning SSRI treatment for non-psychotic depression. In the absence of supporting evidence, and with consideration of other contextual issues, EGAPP discourages use of CYP450 testing for patients beginning SSRI treatment until further clinical trials are completed.

A report on ovarian cancer detection and management evaluated tests for single gene products, genetic variations affecting risk of ovarian cancer, gene expression, and proteomics for CA-125 and BRCA1/2. Although there was no evidence to suggest that genomic tests for ovarian cancer have adverse effects beyond those common to other ovarian cancer tests, i.e. the risks of false-positive results and delayed or inappropriate treatment because of false-negative results, model simulations suggest that annual screening with these tests will not reduce ovarian cancer mortality by more than 50 %.

### NIH's Roadmap Initiative for Medical Research

The National Institute of Health (NIH) supports many programs that facilitate the development of personalized medicine although they are not labelled as such. The NIH plans infused $30 million into its Roadmap initiative in fiscal 2008 as part of an effort to advance and assess several new 'omics areas. Themes of the NIH's "Roadmap Initiative for Medical Research" are:

- New pathways to discovery
- Research teams of the future
- Re-engineering the clinical research enterprise

New Pathways to Discovery focus areas range from molecular imaging and the study of personalized profiles of cell and tissue function at an individual level (leading to better diagnosis and treatment) to studies of biological pathways and networks. This work will help accelerate the achievement of the 2010 predictions of routine genetic testing, personalized medicine and improved quality of patient care.

New initiatives covered under the updated Roadmap involve metagenomics, epigenetics, protein capture, proteome tools, and phenotypic tools. Coordination groups will consider drafting new efforts in pharmacogenomics and bioinformatics. Major new roadmap initiatives that have been approved for funding include a Human Microbiome Project to characterize microbial content in the human body; an epigenetics and epigenomics study that measures changes in gene expression and gene function; and a pilot study for a genetic connectivity map that could help demonstrate linkages between diseases, drug candidates, and genetic manipulation.

### NIH and Personalized Medicine

One US project relevant to personalized healthcare and information-based medicine was initiated in 2003. The National Cancer Institute (NCI) created Cancer Biomedical Informatics Grid (caBIG) to connect cancer research-related elements of data, tools, individuals and organizations and leverage their strengths and

expertise globally. caBIG will help redefine how research is conducted, care is provided and patients and participants interact with the biomedical research enterprise. Participation in this network – based on universal standards for information security and ethical use – means that all stakeholders must adhere to strict security measures for accessing, utilizing and transmitting patient data.

In its funding agreements and its own internal research programs, the NIH is implementing policies to facilitate the exchanges of these research tools and related resources for personalized medicine. NIH's Research Tools Policy defines research tools very broadly, recognizing that the tools may serve as a product in addition to being a research tool. These tools may include cell lines, model organisms, MAbs, reagents, growth factors, databases and computer software. All of these have important uses in development of personalized medicine. Future genomic advances would require a greater collaboration between the NIH, the universities and the industry. This is a new paradigm in the pharmaceutical industry with relation to intellectual property (IP) similar to the situation in case of SNP Consortium. If pharmacogenomic-based tests and associated therapeutics are sold as a package, there may be an opportunity for IP sharing between the upstream and downstream partners in drug discovery and development.

### NIH Collaboration with the FDA

NIH works closely with the FDA for the development of personalized medicine. The NIH will implement this strategy through such efforts as the Therapeutics for Rare and Neglected Diseases (TRND) program. With an open environment, permitting the involvement of all the world's top experts on a given disease, the TRND program will enable certain promising compounds to be taken through the preclinical development phase – a time-consuming, high-risk phase that pharmaceutical firms call "the valley of death." Besides accelerating the development of drugs to treat rare and neglected diseases, the TRND program may also help to identify molecularly distinct subtypes of some common diseases, which may lead to new therapeutic possibilities, either through the development of targeted drugs or the salvaging of abandoned or failed drugs by identifying subgroups of patients likely to benefit from them.

In 2010, the NIH and the FDA announced a new collaboration on regulatory and translational science to accelerate the translation of research into medical products and therapies; this effort includes a joint funding opportunity for regulatory science. Working with academic experts, companies, doctors, patients, and the public, they intend to help make personalized medicine a reality. An example of this collaboration is an effort to identify new investigational agents to which certain tumors, identified by their genetic signatures, are responsive (Hamburg and Collins 2010).

### NIH and Genetic Testing Registry

NIH will address the fact that there is no single public source of comprehensive information about the >2,000 genetic tests that are available through clinical laboratories. In 2010, the NIH, with advice from the FDA and DHHS, started the Genetic

Testing Registry (GTR) as a source of information for healthcare providers and patients about tests and laboratories, and for researchers and regulators to watch the genetic testing industry. GTR includes information about how the tests are used, about their validity and utility, and about how they are accessed. The database, developed by the National Center for Biotechnology Information, is overseen by the NIH's Office of the Director.

### National Human Genome Research Institute

As part of its plans to help translate human genomic research discoveries from the lab into clinical care, the National Human Genome Research Institute (NHGRI) awarded up to $12.8 million to fund new projects to address the challenges that these medical innovations face and the ones they pose. These four 4-year grants, totaling $2.6 million 2013, will fund three research projects that tackle certain problems and questions about the use of genomic information in the clinic and a coordinating center that will help support and organize these projects so that they function as a consortium. The institutions receiving funding include Duke University, the University of Florida, the Icahn School of Medicine at Mount Sinai, and the University of Pennsylvania. As many groups in the US have been studying new ways to take genomic results and implement them into electronic medical records and clinical care, NHGRI is still learning the best ways to do this and putting together a funded consortium of investigators that enables networking to develop best practices and disseminate information.

### National Institute of General Medical Sciences

In 2008, the National Institute of General Medical Sciences (NIGMS) released a strategic plan that outlines its goals over the next 5 years, including an emphasis on continued support for its large-scale research programs such as the Pharmacogenetics Research Network (PGRN), the National Centers for Systems Biology, the Protein Structure Initiative, and the Models of Infectious Disease Agents Study.

PGRN is a group of 12 independently-funded interactive research groups, each with its own focus in an identified area of pharmacogenomic research. The goal of this network is to build a knowledge base of data on how variation in human genes relate to drug responses – Pharmacogenomics Knowledge Base (PharmGKB). It contains both raw and curated information and presents data and information accumulated in the field and contributed by researchers both within and beyond the network.

NIGMS also supports the Electronic Medical Records and Genomics Network (eMERGE), which is a national consortium formed to develop, disseminate, and apply approaches to research that combine DNA biorepositories with electronic medical record (EMR) systems for large-scale, high-throughput genetic research (see section on electronic medical records).

NIGMS' "Investing in Discovery" plan is aimed at guiding the initiatives over the next 5 years, and how it will make strategic investments to maximize the benefits

of the public funds entrusted to it. NIGMS has three central goals it will focus on through the plan, including maintaining a balanced research portfolio, fostering a robust, stable and diverse scientific workforce, and promoting an open dialogue with the scientific community and helping them communicate with the public. NIGMS has allocated up to $10 million per year for as many as three grants to fund the creation of the Systems Biology centers, including one 5-year grant of a total of $14.5 million to Duke University.

Other points of emphasis over the next 5 years will include encouraging development of databases designed to handle genomics and other biomedical research information. NIGMS also plans to continue to support the creation of resources such as sample repositories, databases, interoperable software, and equipment used in exchanging data between various types of researchers. The plan also calls for more inter-institute collaborations and programmatic linkages, including the corollary programs or links to NIH Roadmap initiatives such as the Clinical and Translational Sciences Award through programs like the Medical Scientists Training Program.

In 2009, NIGMS announced that it will grant up to $3 million in the current year to fund one pharmacogenomics knowledge resource that will serve the needs of the entire research community through a NIH funding opportunity. Direct costs for the program are limited to $2 million per year for the PharmGKB over a period of up to 5 years. This program will enable new and renewal applications for an earlier program called the Pharmacogenetics and Pharmacogenomics Knowledge Base. The goal is to support a program that will present complete, comprehensive, and current knowledge in pharmacogenomics, backed by critical datasets, and the most compelling literature. It should support and extend modern research approaches that could help to achieve the goal of using pharmacogenomics to help guide physicians' treatment and therapy decisions. Research topics could include a variety of efforts including comprehensive listings of known genes and gene variants that predict drug responses; definitions of drug responses; current knowledge of genotype-phenotype relationships; accessible views of drug pathways of metabolism, disposition, and sites of action; drug structures, structure-function relationships, and alterations in variants; data-sharing capabilities for addressing questions that can be solved through harmonizing new and existing data sets; possible sources for reagents and models; and other efforts.

In 2010, NIH announced that it will provide $161.3 million in funding to expand a nationwide effort to advance personalized medicine through the use of pharmacogenomics. The 5-year investment in expanding the PGRN will continue funding research into genetic variants linked to responses to a range of medicines for cancer, heart disease, asthma, and addiction and will include new areas of study such as rheumatoid arthritis and bipolar disorder. The new funding will support 14 scientific research projects and 7 network resources, and it will fund development of research methods to study and use pharmacogenetics in rural and underserved populations. These new awards will include funding for deep DNA sequencing, piloting ways to use de-identified medical records to develop pharmacogenomics, expanding collaboration with the Center for Genomic Medicine at the RIKEN

Institute in Japan, and other efforts. The latest funding opportunity announcement from NIH in July 2014 is to encourage pharmacogenetic and epigenetic studies for improving phenotyping in children's pharmacotherapy that will allow individualization of treatment in children.

### National Institute of Standards and Technology

According to a listing in the US Federal Register in 2008, the National Institute of Standards and Technology (NIST) would like genomics, proteomics, and other biomedical researchers to submit ideas about needed advances in personalized medicine, and has asked for white papers detailing. The NIST call is part of a new program asking for input on a number of subjects it has deemed as areas of critical national need, including personalized medicine, and the advice will be used to develop new competitions for funding under its Technology Innovation Program. Researchers could describe needs for advances in genomics and proteomics that could be used to help doctors develop personalized drug treatments and dosages. NIST is not seeking proposals; it is asking for descriptions of the need and associated societal challenge, why government support is needed, the consequences of inaction, and potential technical solutions. According to NIST, personalized medicine, based on genetic, environmental, and metabolic influences on disease, could be a key to addressing the trial and error nature of treatment in the current health care system.

White papers covering personalized medicine could include descriptions of the challenges of cost-effective tools and techniques for genomics and proteomics research, technologies used in identifying biomarkers, drug and vaccine delivery systems, and better methods of integrating and analyzing biological data when it is combined with environmental and patient history information.

### NCI & FDA Collaboration for Clinical Proteomics

An example of application of proteomics to development of personalized medicine is the collaboration between the FDA and the National Cancer Institute (NCI). The new program, called Clinical Proteomics Program, starts with laboratory analyses of cells from tissue samples taken from cancer patients. Normal cells, pre-cancerous cells and tumor cells from a single patient are then isolated using tools that maintain the original protein pattern of the cells. The protein patterns of tumor cells taken from a patient after treatment is analyzed to determine how a particular therapy affects the protein pattern of a cell. Through the Clinical Proteomics Program, the NCI and FDA hope to develop individualized therapies, which are optimal for a particular patient rather than to a population and to determine the effects, both toxic and beneficial, of a therapy before using it in patients. Additionally, the partners hope the program will allow for earlier diagnosis and improved understanding of tumors at the protein level.

**Role of the Centers for Disease Control**

The Centers for Disease Control and Prevention (CDC) has two projects involved in personalized medicine research and oversight, one examining genomics practices in general and the other specifically focused on the environmental aspects of genomics.

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) is a pilot project initiated by the CDC Office of Public Health Genomics. The project's goal is to establish and evaluate a systematic, evidence-based process for assessing genetic tests and other applications of genomic technology in transition from research to clinical and public health practice (see later in this section).

Human Genome Epidemiology Network (HuGENet) is a project aimed at incorporating pharmacogenomics into the practice of public health by assessing the impact of environmental factors on the genetic variations present in large populations. It is a global collaboration of individuals and organizations committed to the assessment of the human genome variation's impact on population health and how genetic information can be used to improve health and prevent disease.

## Role of Academic Institutions in the US

Universities are not directly involved in the development of personalized medicine but research in pharmacogenomics and pharmacogenetics is in progress at several academic centers and nonprofit institutes and it is being applied to patient care. Many of these programs are supported by the US government through NIH. There are some collaborative programs between the academia and the industry that are relevant to personalized medicine. It is beyond the scope of this book to provide an up-to-date directory of all the academic institutes that are involved in personalized medicine. A few of these programs will be described here briefly.

### *Baylor College of Medicine*

Signature Genetics™ (Seryx LLC) is a new tool of personalized medicine introduced at the HealthTexas Provider Network (Baylor College of Medicine), which is designed to assist physicians in customizing drug prescriptions based on an individual patient's unique genetic makeup, as well as identify potential drug interactions. This technology combines the results of genetic testing for a specific patient with scientific knowledge on how genetic variations impact drug metabolism. This is an ongoing service that can be used throughout the patient's lifetime as medications are prescribed.

First, the patient visits the physician's office and has his or her blood drawn and a cheek swab analysis. These samples are sent to a laboratory. Four to six weeks later,

the report, which covers >150 of the most commonly prescribed medications, over the counter drugs and herbal remedies metabolized by CY P450 enzymes, is sent to the physician's office. This report also provides information on drug interactions with these enzymes. Once a patient has been tested and an initial report issued, the physician can easily query Signature Genetics regarding any additional drugs under consideration for that patient. Through this process, the physician receives information specific to both the drug and the patient before actually prescribing the new drug.

### *Coriell Personalized Medicine Collaborative*™

Coriell Personalized Medicine Collaborative™ (CPMC™) is a research study at Coriell Institute for Medical Research (http://www.coriell.org/), which is located on the campus of the University of Medicine and Dentistry of New Jersey in Camden. With five participating centers that include Fox Chase Cancer Center, Cooper University Hospital, Ohio State University, Virtual Health, and Helix Health, CPMC™ is at the forefront of personalized medicine. By combining a functioning biobank facility with modern microarray technology, Coriell has created the ideal environment for this innovative project. CPMC™ is a forward-thinking, collaborative effort involving volunteers, physicians, scientists, ethicists, genetic counselors and information technology experts whose goal is to better understand the impact of genome-informed medicine and to guide its ethical, legal and responsible implementation. CPMC™ seeks to explore the utility of using genome information in clinical decision-making. The project also aims to understand why people often respond differently to treatments and to discover presently unknown genes that elevate a person's risk of cancer and other complex diseases. All volunteers will control their genetic profile. Participants who wish to will be able to view potentially medically actionable information about their genomic profiles through a secure web-browser-based system. A variety of educational material on genomics and medicine will also be provided through streaming video and downloads. This initiative will take an evidence-based approach to determine what genome information is clinically useful while ensuring that patient privacy is vigorously protected. The study seeks to enroll 10,000 participants with an ultimate goal of 100,000 individuals. Coriell is committed to ensuring that the population of CPMC™ study participants resembles the demographics of the Delaware Valley (see following section) as historically, the presence of minority populations in genome-wide association studies has been minimal.

Coriell established a multimillion-dollar Genotyping and Microarray Center – the facility that performs the genome analyses for the CPMC™. This high-capacity facility consists of state-of-the-art equipment and receives samples from laboratories around the world requesting genotyping, microarray and gene expression analysis. The facility also processes up to 2,000 DNA or RNA samples per month. Biobanking repositories provided support to the Human Genome Project, a worldwide program to map the entire human genome, and to the International HapMap

Project, a project providing an efficient tool to identify disease causing genes. The Coriell Institute maintains contracts from the NIGMS and the National Institute of Aging (NIA) to establish and maintain what has become one of the largest cell repositories for the study of genetic and aging-related diseases.

### *Delaware Valley Personalized Medicine Project*

The Delaware Valley Personalized Medicine Project (DVPMP) was established in 2007 with a goal of genotyping up to 100,000 patient volunteers for studies of the use of genetic risk factors in patient care. At the time of its launch, DVPMP enrolled 10,000 participants for the project over the next 3 years and eventually plans to reach 100,000 participants. Partners in the DVPMP include the Fox Chase Cancer Center, Cooper University Hospital, and Virtual Health. In 2008, Coriell Institute for Medical Research (see preceding section) started partnership with Cooper University Hospital, which is the core clinical campus for the Robert Wood Johnson Medical School in Camden, as part of the DVPMP. The collaborators intend to enroll 2,000 Cooper employees and their families in the project.

### *Duke University Medical Center and Genomic Medicine*

The Center for the Advancement of Genomics (TCAG) and Duke University Medical Center (DUMC) started collaboration in 2003 to create the first fully-integrated, comprehensive practice of genomic-based prospective medicine. DUMC and TCAG generated predictive and prognostic data on specific diseases to aid both doctors and patients in the earlier detection and better treatment of these illnesses. The activities included focused research in genomic predictors of diseases; the design of future clinical practice models including personalized health planning; and strategies to tackle ethical and legal issues. Initially funded internally by both organizations, TCAG and DUMC sought outside funding through government grants, foundations and philanthropic donations. This genomic-based medicine collaboration had several goals including creation of a futuristic personalized health plan and medical record including genomic information to predict health risks and outcomes from therapy.

By end of 2014, DUMC's Institute for Genome Sciences and Policy (IGSP), was broken down and restructured into three separate units that include: (1) a translation-focused Center for Applied Genomics and Precision Medicine; (2) a core facility tentatively named the Center for Genomic and Computational Biology; and (3) Duke Science and Society, a free-standing policy and ethics institute that will address a broad range of scientific issues. The facilities and staff from IGSP's core

genomics lab and computational unit will now be consolidated to form the tentatively-named Center for Genomic and Computational Biology, which will try to take genomic and biomarker-driven translational discoveries all the way to the clinic. Teams at the two IGSP centers had previously worked on the gene expression profile discovery effort that eventually led to the CardioDx Corus CAD test, and their pharmacogenetics program was involved in identifying genetic variants that are involved in statin efficacy and safety.

These groups have also conducted research for the US Department of Defense on the use of genomic technologies to predict and diagnose the etiology of infectious diseases. They now have an entire portfolio of gene expression profiles that can distinguish between viral infections, bacterial infections, and fungal infections based solely on host response. Clinical studies are ongoing to demonstrate the usefulness and validity of these profiles. An extensive ongoing program is focused on systems pharmacogenomics and antiplatelet agents using genomics, RNA sequencing, proteomics, and metabolomics to understand the myriad pathways targeted by aspirin and other antiplatelet drugs. These projects also include a family history software platform called MeTree that captures information from patients in their homes and delivers clinical decision reports about their risks for 20 different conditions. The goal is that doctors can use it to decide if their patients should see a genetic counselor, have an additional evaluation, or take a genetic test.

This center will provide core resources, education and training, and computational biology services to scientists from all corners of the campus, including those involved in medical, engineering, environmental, and natural sciences research. Clinical research studies will correlate genomic-based biomarkers with various phenotypes and clinical outcomes, and then try to evaluate their impact patient care. The aim will be to determine the value of genomic and personalized medicine and if should it be adopted and reimbursed and possibly have regulatory approval.

### *Ignite Institute*

The Ignite Institute (http://www.ignitehealth.org/), established in 2009 in Virginia, is a unique non-profit personalized medicine institute, integrating biomedical research, development, commercialization and clinical care. The Institute is the first to apply the latest genomic, biomedical, and technological innovations to enable individualized health care at the community health level, which is the level where most Americans receive care and where medicine needs to be more personalized, efficient and effective. Ignite is built on a collaborative "hybrid" model that includes independent leadership, affiliations with universities and clinical centers of excellence. Research is funded through traditional revenue streams (grants, contracts, philanthropy and licensing revenue) as well as venture capital. The result is a vertically integrated pipeline that moves from discovery to clinical implementation in a shortened timeframe with reduced costs. Inova, a nationally recognized

comprehensive health care network in the National Capital region and an Ignite founding partner, will play a key role in the Institute's development by contributing state-of-the art health care facilities focused on disease prevention and personalized medicine. Inova will be the initial clinical arm for the application of new therapeutics, diagnostics and devices that target the molecular underpinnings of disease. Ignite will house technologies that include genome sequencing systems, a transcriptional profiling facility, a proteomics and metabolomics scanning facility, and facilities for molecular scanning. The institute's research specialties will be cancers, neurological and mental health disorders, diabetes and other metabolic diseases, pediatric diseases, and cardiovascular diseases.

### *Indiana University Institute for Personalized Medicine*

In 2011, Indiana University (IU) used $11.3 million to start the Indiana Institute for Personalized Medicine, which is pursuing genome-based and pharmacogenomics studies in cardiology, obstetrics, pediatrics, cancer, and other areas. The institute will conduct research, train new specialists in personalized medicine, and work to translate its discoveries into more precise therapeutics. The training program will be funded and supported through the new Brater Scholarship in Personalized Medicine. A panel of IU scientists will form an advisory panel that will aid other researches to move their research beyond the laboratory stages. The new institute will also will receive funding and support from the IU School of Medicine and its Department of Medicine, The Indiana University-Purdue University Indianapolis, the Indiana Physician Scientist Initiative, and the Indiana University Melvin and Bren Simon Cancer Center.

In 2012, IU launched a new $150 million, 5-year effort that will use genetics and personalized medicine, among other approaches, to develop new capabilities and translational research projects focused on cancer, neuroscience, and cardiovascular medicine. The funding will be used to support research projects, to recruit new scientists in selected fields, and to retain current scientists at the IU School of Medicine. The new initiative will support research that uses genetic technologies to develop personalized therapies that could be more effective and efficient for individuals and healthcare providers, and also will fund translational projects and clinical trials. One of the new initiatives will enable the IU and Bren Simon Cancer Center to achieve NCI's 'comprehensive' status, and will involve the recruitment of leading cancer researchers and the expansion of cancer clinical trials. Under the cardiovascular initiative, the partners will develop a cardiovascular genetics program and recruit a scientist in the field, and will develop a comprehensive program for the study and treatment of heart failure across the lifespan. The neuroscience research program will involve research into a wide range of brain injuries, neurodegenerative disorders, and neurodevelopmental disorders.

## *Institute of Medicine's Role in Personalized Medicine*

The Institute of Medicine (IOM) is an independent, nonprofit organization that works outside of US government to provide unbiased and authoritative advice to decision makers and the public (http://www.iom.edu). In 2010, the IOM started a new committee, Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials, that will look at predictive 'omics-based tests and eventually issue a report and recommendations on how to determine when these tests are fit for use as a basis for designing clinical trials, for stratifying patients, and measuring patient response in clinical trials – activities relevant to the development of personalized medicine. It expects to release its first report in Spring of 2012. Although the specific details of the committee's tasks remain uncertain, its charge is to review the published literature to identify what criteria will be appropriate for evaluating tests based on 'omics tools, including genomics, epigenomics, proteomics, and metabolomics tests. After conducting this review, the committee will recommend an evaluation process for when these tests are fit for use in designing and stratifying trials and measuring patient response. The group also will identify which criteria are important for the analytical validation, qualification, and utilization components of the test evaluation process. After developing those evaluation criteria, the committee then will apply them to three cancer clinical trials conducted by researchers at Duke University. For example, one of these Duke studies involved partnering with Eli Lilly and used Affymetrix gene-expression data with corresponding drug-response data to provide personalized chemotherapy regimens for two types of lung cancer.

Although how the committee will apply these criteria has not yet been determined, several approaches may be used. The committee may assess the analytical methods used to generate and validate the predictive models, examine how the source data were used to develop the test and how the predictive models were generated, or evaluate the use of predictive models in clinical trials.

## *Jackson Laboratory for Genomic Medicine*

In 2011, The Jackson Laboratory (Jax, Bar Harbor, Maine) announced plans to build a genomic medicine research center in Connecticut and is working with the state's governor and the University of Connecticut (UConn) to ask the legislature to help fund the building and its operations. Jax Genomic Medicine would be located on the UConn Health Center's campus in Farmington, and would employ 300 people over the first 10 years and a total of 600 within 20 years. Jax Genomic Medicine would combine Jax's genetics and genomics expertise with the clinical and biological capabilities of Connecticut's institutions, including UConn and Yale University. Specialty areas for the new lab could include cancer, aging, genetic disorders, metabolic diseases, and others. Space would be dedicated to the translation of new applications such as diagnostics and computational services into commercial products. Jax wants to help the UConn center to expand its faculty and to advise the state's economic development agencies to identify the best industrial and biotech partners in personalized medicine.

## *Johns Hopkins Center for Personalized Cancer Medicine Research*

In 2011, The Johns Hopkins Kimmel Cancer Center received a $30 million donation from the Commonwealth Foundation for Cancer Research to fund a new center that focuses on genomics and personalized oncology research. The Center has initially undertaken pilot projects focused on DNA mutations and epigenetic alterations in cells. Researchers at the center will study genomic and epigenomic factors that affect leukemia and lung cancer patients' responses to treatment and develop tests for early detection of various types of cancer. The long-term aim will be the development of individualized immunotherapies such as cancer vaccines and pharmacogenomics-based treatment tools based on genetic discoveries.

## *Mayo Clinic's Centers for Individualized Medicine*

The Mayo Clinic (Rochester MN) was one of the first academic hospitals in the US to start personalized medicine programs. The clinic has a range of resources, including genome sequencing, proteomics, and gene expression facilities. Translational programs focus on biomarker discovery, clinical genomics, epigenomics, pharmacogenomics, and the microbiome. Infrastructure programs include a medical genomics facility, biorepositories, bioinformatics resources, as well as bioethics and education/training. Several projects in various therapeutic areas such as management of hypertension and CLL have already applied a personalized medicine approach. The Mayo Clinics in Arizona and Forida also have personalized programs.

The Mayo Clinic Center for Individualized Medicine and Whole Biome are collaborating to develop microbiome-targeted diagnostics. The initial focus will be on women's health, and in particular preterm labor. Mayo Clinic plans to develop a test to enable the early indication of preterm labor.

In 2007, the Mayo Clinic, in collaboration with the IBM, set up Mayo Clinic Life Sciences System, which is designed to include detailed digitalized genetic information of patients. The trial helped physicians at the Mayo Clinic to work out the best way to store a person's genetic code, develop procedures to explain the information to patients, and direct their medical care. Questions that arise are: who is going to store the information, how is it going to be stored securely, who has access, and what is going to happen to the information that the patient might not want to know about? There are some significant ethical and privacy issues, which are more difficult to solve than storing the information.

The Mayo Clinic launched a pilot study early in 2012 as part of a move towards an era of "proactive genomics" that puts modern genetics at the center of patient care. The project help managers at the clinic decide whether it makes sense to read and store a patient's WGS to start with instead of ordering single genetic tests as and

when the need arises. This is feasible as the cost of sequencing a person's whole genome has fallen so rapidly that it is now comparable to the price of a single gene test. Patients who join the study will have either their WGS read or a subset of genes that are linked to diseases. Another group will be tested for 83 genes that govern how the body metabolizes drugs. Through discussions with physicians and counsellors, patients who have WGS will decide how much genetic information they want to know. Most patients are expected to want to learn only about genetic risk factors that lifestyle changes or medication can influence. Physicians will keep track of the patients and their prescriptions to see whether WGS or more limited genetic testing, benefits them or reduces the costs of their treatment. The shift towards WGS is expected to become more valuable as physicians and scientists piece together how multiple genes influence disease and how the body reacts to drugs. WGS will not only give a fuller picture but also enable drawing of complex interaction pathways, which is not possible by going after only selected genes.

The Mayo Clinic launched a new clinical center in Jacksonville, Florida in 2013 that uses genomic technologies to tailor treatments to individual patients. Genomics scientists, genetic counselors, bioinformatics experts, and bioethicists, will work with physicians to determine whether specific patients are good candidates for treatments guided by genetic testing. This multidisciplinary group will provide consulting for cancer patients who have seen standard treatments fail and for patients with "diagnostic odyssey" cases, disorders that are complex or difficult to diagnose but which appear to be genetic in origin.

## *Mt. Sinai Medical Center's Personalized Medicine Research Program*

In 2007, the Mount Sinai Medical Center in New York received a $12.5-million donation from Andrea and Charles Bronfman Philanthropies that it will use over 10 years to start the Charles Bronfman Institute for Personalized Medicine. The research center is studying personalized medicine, and the medical center will use the funds to start "an institution-wide biobank" and a "translational biomedical informatics center." The grant will also go toward what will become a $30-million personalized medicine initiative. The Institute will bridge the gap between genomics research and clinical patient care in the area of personalized medicine. The Personalized Medicine Research Program will develop and provide essential core technologies that will enable genome-wide analysis of genetic variation and function in human DNA, and quantitative biology at the single molecule level for large-scale studies of genetic associations and predictive biomarkers. Access and training in these resources will be critical to overcoming current research infrastructure barriers that limit our disease-oriented research centers in deciphering the genetic underpinnings of, and developing personalized approaches to, complex diseases.

### *New York Genome Center*

New York Genome Center (NYGC), a non-profit center, will focus on sequencing, bioinformatics, and genomic medicine. It is comprised of 11 collaborators, mostly from New York City but also from institutions in other states. Through the unique collaboration, scientists and physicians will share clinical and genomic data on a large scale in studies aimed at identifying and validating biomarkers, understanding the molecular basis of diseases, and speeding up the development of new diagnostic and therapeutic technologies. It is using an initial $125 million investment to build the 120,000 square-foot center in Manhattan to begin operations. NYGC is outfitted with technology from Illumina, one of its founding members. Roche has also joined Illumina as a corporate member. The other founding members include Cold Spring Harbor Laboratory; Columbia University; Cornell University/Weill Cornell Medical College; Memorial Sloan-Kettering Cancer Center; Mount Sinai Medical Center; New York-Presbyterian Hospital; New York University/NYU School of Medicine; North Shore-LIJ Health System; The Jackson Laboratory; The Rockefeller University; and Stony Brook University. The Hospital for Special Surgery is an associate founding member. The partner institutions serve >5 million patients and offer scientists a broad and diverse range of genetic variation that would be difficult to find in any other single region. The center's core components will be its sequencing capabilities and its bioinformatics labs, but it also will be home to a CLIA-certified lab for clinical research, an innovation center for developing new genomic technologies, educational and training programs in genomics, and a philanthropic unit.

New York City is the largest concentration of medical and academic research anywhere in the world. The diversity of the patient populations here is really important. It provides a place where new products and tests can be developed for a variety of ethnic groups and age groups for which different drugs and approaches might be appropriate.

### *P4 Medicine Institute*

The P4 Medicine Institute (http://p4mi.org/) is driving innovative approaches to disease prevention and maintenance of health and wellness by applying systems biology to medicine and care delivery. P4 stands for predictive, preventive, participatory and personalized medicine. P4Mi was co-founded in 2010 by the Institute for Systems Biology and the Ohio State University Medical Center, which is developing more specific, cost-effective treatments for patients, creating new technologies and tools that will define wellness at a deep molecular level, and empowering individuals to take an active role in their health care. P4Mi is now joined by PeaceHealth, a Washington-based not-for-profit Catholic health care system, with major medical centers and laboratories in Alaska, Washington and Oregon with approximately 15,000 employees.

## *Personalized Medicine Partnership of Florida*

In 2012, Sanford-Burnham Medical Research Institute, Moffitt Cancer Center, and Florida Hospital launched Personalized Medicine Partnership (PMP) of Florida that will use molecular and genomic tools to develop new technologies for preventing and treating cancer, cardiovascular diseases and metabolic disorders such as obesity, and diabetes. The partnership will engage Sanford-Burnham's research expertise and genomics and metabolomics technologies, Moffitt's biospecimen bank, data warehouse, and genome mapping capabilities, and Florida Hospital's patient population and clinical research capacities.

A particular focus for PMP Florida will be to use Moffitt's Total Cancer Care, a treatment program that involves the mapping of more than 30,000 genes in a tumor and then using that data to develop individual therapies that also include other medical, nutritional, and psychological considerations. The partners have complementary areas of expertise and experience. PMP Florida also seeks to attract industry clients from the pharmaceutical and biotech sector who can utilize this unique resource for discovery and development of new advances in health care. This partnership will demonstrate how personalized medicine discoveries made in research labs will improve health care in hospitals, clinics, and medical offices in Florida and across rest of US.

## *Partners Personalized Medicine at Massachusetts General Hospital*

Since its founding in 2001, the mission of Partners Personalized Medicine has been to promote genetics and genomics in research and medicine and to help realize the promise of personalized genetic medicine by accelerating the integration of genetic knowledge into clinical care. It provides personalized medicine services to >4,000 patients and their physicians each year through >100 targeted genetic tests, WGS, and WES. Further details can be seen on the web site: personalizedmedicine.partners.org/.

### Personalized Oncology

In 2009, oncologists at the Massachusetts General Hospital started to personalize cancer therapy. They read the genetic fingerprints of nearly all the new patients' tumors in a strategy designed to customize treatment. They will search for abnormalities carried on major cancer genes that can predict whether drugs already available or in development might be effective against a particular patient's cancer. High throughput techniques are being used for sequencing 5,000–6,000 patients a year, replacing labor-intensive techniques that had been used only selectively for a handful of cancers. The focus is more on the genetic profile of a tumor and less on

whether it is in the lung, breast, or prostate. The genes inside the malignancy are considered to be more important than the location of the cancer. The testing could be especially useful for patients with rare cancers, usually neglected by cancer researchers or pharmaceutical companies, as they may share genetic signatures with more common tumors already being successfully treated. One limitation is the cost as the hospital charges $2,000 for the test and it may not be covered by the health insurance companies.

### *Personalized Oncology at Oregon Health & Science University*

In 2010, Oregon Health and Science University's (OHSU) Knight Cancer Institute (Portland, OR) started to recruit new researchers and launch initiatives focused on exploring the cellular pathways through which cancer has been shown to grow. It manages >1,000 research projects, and conducts ~400 clinical trials every year. OHSU Knight Cancer Institute plans to more than double the current capacity of 20,000 samples of its tissue bank as well as expand its molecular laboratory service by combining the four existing labs on campus under the umbrella of Knight Diagnostic Laboratory, which will maintain its cancer focus but also focus on rare diseases. Through these efforts, the institute will develop a new model for patient care designed to tailor treatment plans toward the tumor biology of each individual patient. The institute envisions genotyping tumors so patients can be linked to new therapies or, if deemed appropriate, clinical trials. The Institute is also promising to overhaul its clinical molecular lab operations, which can map the genomes of patients to determine the appropriate treatment for their cancers. The main set of tools and technologies to be acquired will be for next-generation sequencing. The aim is to advance personalized cancer therapy.

### *Southeast Nebraska Cancer Center's Personalized Medicine Network*

In 2005, the Southeast Nebraska Cancer Center (Lincoln, Neb) was awarded $1.5 million in US Department of Defense (DoD) appropriations to support a network and database of cancer patients' tissue samples. The center, will be part of the DoD's National Functional Genomics Center, and will use the funding to create a network to collect cancer tissue samples and to follow the patients' progress through therapy, which would be merged into a national database. This large-scale effort combines government, academic and private-sector resources. The program also uses "systems biology" approach, which bring together advanced science in pharmaceuticals, molecular biology, genetic screening, bioinformatics and other technologies. The system will allow personalized cancer treatment decisions based on patients' molecular profiles. This research will help us identify genomic sequence changes

associated with cancer in individual patients. The center's aim for the future is that a physician can run a simple test on a small tumor sample and use a quick genetic analysis to tailor the best therapy for the patient as an individual.

## *Stanford Center for Genomics and Personalized Medicine*

In 2010, Stanford University's School of Medicine created a Center for Genomics and Personalized Medicine designed to integrate genomics with medicine, as well as draw on collaborations between Stanford's basic scientists and clinical researchers, and on technologies developed in the Silicon Valley. The center l promotes personalized medicine by building on research from the sequencing of the genome of Stephen Quake, by using Heliscope single molecule sequencer and showing the potential for use of the information obtained in assessing personalized disease susceptibility and responsiveness to drugs. The center blends highly efficient, rapid sequencing technology with the research and clinical efforts of experts in genomics, bioinformatics, molecular genetic pathology and even ethics and genetic counseling to bring advances from the laboratory to the patient. The center's sequencing facility is already operating with new equipment estimated to increase its sequencing capacity by about fivefold while significantly reducing the cost.

### Stanford's Pharmacogenetics Research Knowledge Base

Pharmacogenetics Research Network and Knowledge Base maintain PharmGKB (http://pharmgkb.org/) at Stanford University. This program is funded by a grant from the NIH and has the support of the academia, the regulated industry and regulatory agencies such as the FDA. This is an integrated resource about how variation in human genes leads to variation in our response to drugs. Current studies include the gene-drug effects associated with asthma, cardiac problems, and cancer; the roles of genetic variability in drug response in ethnic populations; genetic differences and estrogen receptors; and the effects of gene variability on membrane transporters, which interact with one-third of all prescription drugs. Consumers of the new information will include pharmacogeneticists interested in the interaction of particular drugs with phenotype and statisticians who are more broadly tackling the phenotype-genotype problem. Genomic data, molecular and cellular phenotype data, and clinical phenotype data are accepted from the scientific community at large. These data are then organized and the relationships between genes and drugs are then categorized into the following categories:

- Clinical outcome
- Pharmacodynamics and drug responses
- Pharmacokinetics
- Molecular and cellular functional assays
- Genotype

### *UAB-HudsonAlpha Center for Genomic Medicine*

The University of Alabama at Birmingham (UAB) School of Medicine and the HudsonAlpha Institute for Biotechnology started a partnership in June 2014 to create a new research center that will accelerate genomics discoveries and translate them into clinical practice. UAB-HudsonAlpha center will focus on using genomic and molecular approaches to study etiology as well as progression diseases, and will incorporate research findings into clinical studies aiming to predict and diagnose diseases for developing personalized approaches to treatment. It will formalize an ongoing collaborative relationship that has combined HudsonAlpha's genomics tools, know-how, and infrastructure with UAB's academic research and clinical medicine capabilities. The center will include a cross-section of scientists and physicians from UAB and HudsonAlpha working in multiple disease areas that will integrate genomic information into their specialties. The center also will conduct large scale genome sequencing to discover genes that are involved in diseases, and will start a program to teach researchers and physicians how to incorporate genomic information into their studies or clinical practices, and to train graduate students.

### *University of Colorado's Center for Personalized Medicine*

In 2014, University of Colorado Health (UCHealth) announced a plan to use $63 million to create a new personalized medicine center that will use genomic testing and bioinformatics in clinical care and in research programs to treat patients and improve outcomes. UCHealth will contribute $26 million and its partners at the UC School of Medicine, University Physicians Inc, and the Children's Hospital Colorado will provide the remainder of the center's funding. A new Division of Personalized Medicine will be created within the School of Medicine. University of Colorado-affiliated institutions include the Colorado Health Medical Group; the Medical Center of the Rockies; Memorial Hospital Central; Memorial Hospital North; Poudre Valley Hospital; and the University of Colorado Hospital. The center will be housed at the UC Anschutz Medical Campus in Denver, but it will serve patients and clinicians at UCHealth and Children's Hospital Colorado locations spread around the state.

The center will provide genetic and molecular testing services and consultations, and it will house a DNA bank resource that will sequence and store samples from around the region for use in treatment and research. These services will include tests to predict disease risk, to assess cancer patients' responses to treatment, and to identify the correct drugs and dosages for patients. Identity of the samples in the DNA bank will be encrypted. An analytics department at the center will analyze and research the molecular and genomic data from the tests, and the center will hire

more clinicians, geneticists, and counselors specializing in hereditary cancer. The Colorado Molecular Correlates laboratory at the campus will handle the samples and testing, and is expanding to scale up for the increased number of submissions. Some personalized medicine and genetic counseling services are already being offered, and the center will expand upon these.

### *UNC Institute for Pharmacogenomics and Individualized Therapy*

Institute for Pharmacogenomics and Individualized Therapy (IPIT) is a collaborative effort between the University of North Carolina (UNC) Eshelman School of Pharmacy and the School of Medicine, Gillings School of Global Public Health, and the School of Nursing, with substantial support from the Lineberger Comprehensive Cancer Center and the Carolina Center for Genome Sciences. Leadership in these key areas of research is fostered by the creation of contiguous office and laboratory space that bolsters collaborations and the development of comprehensive research investigations and treatment tools. The mission of IPIT is to employ an interdisciplinary approach to tailor therapies and enable the delivery of individualized medical practice. IPIT also offers the services of facilities in molecular genomics, cellular phenotyping, and pharmacoinformatics to add to the excellent core facilities already existing at UNC (http://ipit.unc.edu/). Pharmacogenetics for Every Nation Initiative (PGENI) at IPIT has a mission is to help developing countries use genetic information to improve their drug dosing decision-making process. The cost of individual genetic testing will be prohibitive in these countries for some time, therefore, the PGENI's strategy is to integrate pharmacogenomics into public health decision making without placing an extra burden on sparse healthcare funds and technology infrastructure. IPIT is using DMET™ Plus biomarker panel (Affymetrix), which enables genotyping of the largest and most comprehensive set of key functional drug metabolism alleles within a single panel. The data gathered by PGENI researchers will help them analyze populations in the developing world for their response to most commonly used medications. The program will initially focus on Jordan, Mexico, India, China, Brazil, Ghana, and South Africa, and the university eventually plans to expand to more than 100 countries.

### *Wisconsin Genomics Initiative*

In 2008, four Wisconsin-based research institutions started collaboration to form the Wisconsin Genomics Initiative with a focus on personalized healthcare research. The collaborators include the Marshfield Clinic, the Medical College of Wisconsin, Department of Public Health, and the University of Wisconsin-Milwaukee. The institutions will combine resources to conduct research on predicting individual susceptibility to disease, targeting personalized treatments, determining how

patients respond to specific treatments, and disease prevention. One of the participants, Marshfield Clinic, is home to the Personalized Medicine Research Project, a population-based genetic research project that has so far collected DNA and medical records from ~20,000 persons.

## Role of Healthcare Organizations

Initially, Healthcare organizations did not show much interest in implementation of personalized medicine. The first example in the US is the Signature Genetics program in Texas. Major health insurance companies such as Blue Cross and Blue Shield are now interested in this topic. Other healthcare organizations are collaborating with universities in developing personalized medicine. A recent example is in Canada.

In November 2014, Hospital for Sick Children (SickKids), University Health Network, and University of Toronto announced the creation of the Ted Rogers Centre for Heart Research, funded by a private donation of $115 million. Among the methods that the new center will use are genomic technologies to decode the genetic underpinnings of cardiac disease. The center will integrate research in genomic medicine, stem cells, and bioengineering, to develop personalized disease management. Research will cover the entire human life span, from childhood to adulthood, with each partner focusing on improving a particular aspect of cardiac health. SickKids will perform cardiogenomic studies to improve prediction of cardiac disease before clinical manifestations and to provide personalized medicine to both children and adults. In 2012 it partnered with Life Technologies (now part of Thermo Fisher Scientific) to establish the Centre for Genetic Medicine, with a goal of sequencing 10,000 pediatric genomes per year. University Health Network will use bioinformatics to translate discoveries into advances in healthcare delivery and development of personalized medicine. University of Toronto's Institute for Biomaterials and Biomedical Engineering will apply stem cell technology and novel approaches in cellular and tissue engineering to the cutting-edge science of regenerating heart muscle, coronary vessels, and heart valves. Research at the University of Toronto will also focus on how genetic, cellular, and molecular signaling networks function as the heart develops. The research center will bring together more than 30 expert scientists and clinicians from the three institutions, as well as up to 80 graduate students, postdocs, and clinical fellows.

## Role of the Medical Profession

Substantial advances are being made in genomics and the results are beginning to play an important role in the practice of general clinical medicine. It is important for physicians involved in clinical practice to become more aware of emerging genomic

data and participate in integrating medical genomic information into clinical practice. Professional organizations such as the American Medical Association (AMA) have an important role.

## *The American Medical Association and Personalized Medicine*

The AMA oversees medical nomenclature critical to any field of medicine as it enters the marketplace. The AMA would be involved with personalized medicine through its Current Procedural Terminology codes. CPT codes are the most widely accepted medical nomenclature used to report medical procedures and services under public and private health insurance programs. CPT is maintained by the CPT Editorial Panel, which meets three times a year to discuss issues associated with new and emerging technologies as well as difficulties encountered with procedures and services and their relation to CPT codes.

## *Medical Education*

As knowledge in molecular genetics and cell biology accelerates, the biomedical community is finding it increasingly hard to harness the explosion of new information and translate it into medical practice. Biomedical scientists should be trained to apply new biological knowledge to human health. A better understanding of medicine also can guide scientists in research directions that are most likely to benefit the diagnosis and treatment of human disease.

There is a growing need to incorporate the increasing body of knowledge of pharmacogenetics and pharmacogenomics in the standard curriculum of medical schools, so that the next generation of clinicians and researchers will be familiar with the latest developments in these areas, and will be capable of providing patients with the expected benefits of personalized medicine. As a first step, and in recognition of such emergent needs, the graduate school of the Sackler Faculty of Medicine at the Tel-Aviv University in Israel introduced a course in 2002 titled 'Introduction to Pharmacogenomics: Towards Personalized Medicine' for graduate and undergraduate students with a basic background in pharmacology and human genetics.

As personalized medicine is being developed by the pharmaceutical industry, there should be a parallel education of the public and physicians on these issues. The present generation of physicians does not have any formal education in molecular medicine and this can be remedied by continuing education. This can be accomplished by conferences and symposia sponsored by the industry. For the busy physician who is unable to attend such conferences, the Internet educational programs offer an alternative. Extra courses need to be incorporated in the medical curricula and the pharmaceutical industry may invest in endowing chairs and supporting courses on clinical pharmacology that include pharmacogenetics, pharmacogenomics and personalized medicine. The ethical objection to involvement of pharmaceutical

companies that occurs while conducting symposia for pharmaceutical products does not apply to industrial sponsorship of education in techniques on the frontiers of modern medicine. Apart from the education of the physicians, active steps are needed to encourage the incorporation of personalized medicine into clinical practice.

The mere availability of new tests, new knowledge, and individually tailored medicines is no guarantee that these will be incorporated in clinical practice. The ability and willingness of physicians to adopt personalized medicine into practice is an important factor in realizing its potential benefits. However, studies in the field of innovation adoption as well as physician clinical reasoning processes indicate that all physicians do not incorporate new techniques into their practices at the same rate and some fail do so. The concern that personalized medicine will not be readily or proficiently integrated into practice is suggested by evidence that primary care physicians have not significantly increased referrals for genetic services, nor have they increased identification of candidates who are appropriate for genetic testing.

An understanding of the physicians' clinical reasoning processes or habits of diagnostic decision making may help to identify and remove the barriers to assimilating genetics related innovations into clinical practice. Focused training and educational materials need to be developed to address not only the substance of new information but also the assumptions and diagnostic strategies that drive the practice of medicine.

### *Off-Label Prescribing and Personalized Medicine*

The term "off-label" is used when a drug or medical device is used to treat a disease or condition not listed on its label, or used in such a way that's not outlined in the label, it is said to be used off-label. This off-label use is also sometimes referred to as extra-label use, nonapproved use or unapproved use. Off-label prescription is a common practice because new indications for approved drugs may not be tested in clinical trials due to heavy cost involved or may be in the long process of approval. However, policy forces inside the US government discourage the use of genomic technologies to help physicians make off-label prescribing decisions. Physicians will not be able to always wait for FDA to approve a new label for every one of their patients, and drug companies will not be able to conduct a trial to explore every possible contingency. In the future, personalization of care could mean much more off-label use of new medicines, guided by the latest literature, at least until the regulatory approaches are able to fully adapt to a different paradigm where treatment is highly specific to individual patients.

## Role of Patients

Educated patients with an interest in healthcare have easy access to information on conventional medicine and new trends based on scientific advances. Patient attitudes will be an important factor in the development of personalized medicine.

The Patient-Centered Outcomes Research Institute (PCORI) was created in the US to conduct research for providing information about the best available evidence to help patients and their health care providers make more informed decisions. Research topics supported by PCORI include personalized medicine (http://www.pcori.org/).

## *Public Attitude Towards Personalized Medicine*

It can be anticipated that the public, particularly in the US, would be receptive to the concepts of personalized medicine as it would improve health care. However several issues need to be addressed. The primary one is the education of the public. There are other issues such as public attitudes towards genetic testing that will affect the development of personalized medicine.

In 2007, a federal and private joint study started to investigate the attitudes of young adults toward undergoing genetic testing for common diseases, and about how they would use information provided by such tests. The study, called the MultiPlex Initiative, aims to understand how the development of personalized medicine might be affected by the attitudes towards genetic testing held by individuals aged 25–40 years. The study was conducted by the NHGRI, the NCI, the Group Health Cooperative in Seattle, and the Henry Ford Health System (Detroit, MI). The MultiPlex Initiative will study 1,000 individuals in the metropolitan Detroit area and will include tests based on 15 genes linked to type 2 diabetes, coronary heart disease, hypercholesterolemia, hypertension, osteoporosis, lung cancer, colorectal cancer, and malignant melanoma. According to the NIH, the study will look into what types of individuals are and are not interested in receiving genetic testing, what influences their decisions, and how these individuals interact with the health care system. It also aims to understand how people who decide to take the tests will interpret and use the results in making their own health care decisions in the future. The initiative will provide insights that will be a key to advancing the concept of personalized medicine. The NHGRI's Bioinformatics and Scientific Programming Core designed an innovative system for data collection and analysis for the study. The Center for Inherited Disease Research, operated by the NIH and the Johns Hopkins University, will handle the genetic testing for the study.

According to a 2008 telephone survey of public attitudes about biomedical science, a majority of Americans support advancing genetics research and genetic testing, although more than one third are concerned about the safety guarantees of such science. According to Virginia Commonwealth University's Life Sciences Survey 2008, 80 % of Americans favor making genetic testing easily available to all who want it, approximately the same number who felt that way in 2001 and in 2004. Americans also see genetics as playing a role in their lives, with 45 % of adults saying that they have a disease or a medical condition that is strongly related to genetic factors, an increase of 7 % over the 2007 survey. Among the 80 % who support making genetic tests easily available to all who want them, 38 % were somewhat in favor of such access and 42 % were strongly supportive. The margin of error for the survey is +/− 3.8 percentage points.

A survey conducted in 2013 by GfK Bridgehead, an international market research firm, aimed to gauge consumer perspectives on personalized medicine. The surprising finding was that only just more than a quarter of people have heard of the term 'personalized medicine'. Additionally, many people think that such tailored treatments will lead to a rise in healthcare costs, which was the main thing they wanted to learn about personalized medicine. Of those that knew the term 'personalized medicine,' only a handful could describe it correctly; 4 % said it dealt with medicine based upon a patient's genetic or genomic makeup. About half of the respondents, though, were interested in genetic testing, particularly those with past cancer diagnoses (~80 %). Those who were interested were materially different than those who were not as follows:

- On average, those who were interested were more educated, had better health, and saw the value of personalized medicine impacting disease prevention
- In contrast, those who were not interested in genetic testing were less comfortable with handling genetic data, visited doctors less frequently, and saw the value of personalized medicine playing out once a disease is diagnosed.

A study has shown that pharmacogenetic testing may serve as another tool to boost patients' confidence in the safety and efficacy of prescribed medications (Haga and LaPointe 2013). It has a positive impact on medication-taking behavior and reduces non-compliance which is well-known in patients with chronic conditions, and is associated with significant morbidity, mortality and health-care costs.

## Role of Genetic Banking Systems and Databases

Genetic databases will be an important source of information for development of personalized medicine. Most of these are covered under the term "biobanks".

### *Role of Biobanks in Development of Personalized Medicine*

A biobank is any collection of biological samples and associated clinical data. There are biobanks for diagnostics as well as therapeutics. With the advent of genomic era, the traditional purpose of a biobanks, such as blood bank is for storage and distribution of blood, has not been expended to include research into specific populations or specific diseases. These facilities are important for the development of personalized medicine. However, serious ethical issues have been raised about biobanks and considerable work will be required to resolve the concerns about privacy and consent.

#### UK Biobank

The UK Biobank project is the largest resource for the study of the role of nature and nurture in health and disease. The project is funded by the Medical Research Council of UK, the Wellcome Trust biomedical research charity, the Department of

Health and the Scottish Executive. Up to 500,000 participants aged between 45 and 69 years will be involved in the project. They will be asked to contribute a blood sample, lifestyle details and their medical histories to create a national database of unprecedented size.

This information will create a powerful resource for biomedical researchers. It will enable them to improve our understanding of the biology of disease and develop improved diagnostic tools, prevention strategies and personalized treatments for disorders that appear in later life. UK Biobank will seek active engagement with participants, research users and society in general throughout the lifetime of the resource. Data and samples will only be used for ethically and scientifically approved research. Strong safeguards will be maintained to ensure the confidentiality of the participants' data.

**Biobanking and Development of Personalized Medicine in EU**

The Biobanking and Biomolecular Research Infrastructure (BBMRI, www.biobanks.eu), which started the preparatory phase in 2008, will pool all of the major biobanks in Europe. Together these represent approximately 12 million blood, body fluid, and tissue samples. In the following 2 years, BBMRI will try to create the preconditions to make the biological materials and data available, as well as to standardize the analyses platforms and sample preparation. The project not only includes the organization and funding of the EU biobank, but also aims to establish a complete resource for EU life scientists, including a variety of affinity binders and molecular tools as well as a biocomputing infrastructure that will work with standardized protocols, making data generated from those materials more comparable. The BBMRI was selected for FP7 funding as one of six EU infrastructure projects that are supposed to benefit all EU researchers. It is still awaiting the grant agreement from the European Commission.

No single biobank can be large enough to generate statistically significant data of specific disease subtypes and it takes more than a few dozen or even hundreds of cases in well-defined diseases to correlate disease history or patient response to a certain therapy and to biomarkers. The 134 associated partners of the BBMRI could together provide about 2.4 million samples from population-based biobanks, and a further 10 million from disease-orientated biobanks. The project will seek to overcome the current fragmentation in biobanking, and could also become an interesting tool for the biopharmaceutical industry when validating biomarkers. The information generated from BBMRI will be useful for the development of personalized medicine.

The joint initiative, which will tie together Europe's top research groups across almost every area of molecular and cell biology, also has a political dimension. Because the protection of the data obtained from biological samples continues to be a sensitive subject, the initiative will need to conform to all the national legislations involved. For that purpose, the partners plan to establish a widely-accepted and harmonized set of practices in line with the heterogeneous landscape of European and national regulations. For instance, the protocol to be added to the Convention of Human Rights, which was approved by the EU Council in 2007 and has now been

sent out to member nations for ratification, states that the confidentiality of the information obtained through diagnostic, predictive and pharmacogenetic tests of the samples must be assured. The researchers will have to find procedures that assure a high degree of data protection while simultaneously allowing use of the patient data to acquire deeper insights into the causes of various diseases.

**Lausanne Institutional Biobank**

The Lausanne Institutional Biobank was designed as an integrated, highly versatile infrastructure to harness the power of emerging omics technologies and catalyze the discovery and development of innovative therapeutics and biomarkers to advance personalized medicine (Mooser and Currat 2014). Since January 2013, inpatients admitted at Lausanne CHUV University Hospital have been systematically invited to provide a general consent for the use of their biomedical data and samples for research, to complete a standardized questionnaire, to donate a 10 ml sample of blood for future DNA extraction and to be re-contacted for future clinical trials. Over the first 18 months of operation, 14,459 patients were contacted, and 11,051 accepted to participate in the study. This initial 18-month experience shows that a systematic hospital-based biobank is feasible with a strong engagement in research from the patient population and the need for a broad, integrated approach to personalized medicine. Potential applications include:

- Discovery of genetic as well as molecular basis of diseases and response to interventions
- Validation of genetic or other biomarkers
- Proof-of-concept studies for investigational therapeutics on carriers of selected genetic mutations
- Genetically enriched phase IIb/III studies on selected participants of biobank
- Predictive and preventive medicine

## *CARTaGENE for Biobanks in Canada*

In 2007, The Canadian government and the government of Québec announced a plan to pump CA$34.5 million (US $31.9 million) into a human genomics consortium. The Public Population Project in Genomics, or P3G, could receive as much as CA$64.5 million when funds from other partners are counted. The primary aim of the Montreal-based P3G consortium is to foster "collaboration between researchers and projects in the field of population genomics." The group also includes the ongoing CARTaGENE project. One of the major projects will be the creation of a large bio-bank, which will comprise data from 20,000 residents of Québec between the ages of 40 and 69. The infrastructure will function as a precursor for the development and testing of standards for large biobanks in Canada.

## Role of Bioinformatics in Development of Personalized Medicine

Bioinformatics is the use of highly sophisticated computer databases to store, analyze and share biological information. This is a new discipline at the interface of computer sciences and biology. The massive amount of information generation by the Human Genome Project, detection of SNPs and proteomic data would require bioinformatic tools for cataloguing and analysis of information. Personalized medicine is often referred to as information-based medicine.

Bioinformatics tools will integrate various technologies and sources of information to facilitate the development of personalized medicine and informed therapeutic decision-making by the physicians as shown in Table 20.3.

A large amount of information on the function and interaction of human genes has accumulated from functional genomic projects. This information is valuable with respect to molecular diagnostics. Advances in bioinformatics have helped in lowering the cost of individual genetic screening. The speed with which individuals can be screened for known genetic conditions and variations has increased. Bioinformatics has provided a large number of software tools for classifying expression profiles and reduction of dimensions of data followed by regularized classification, which can

**Table 20.3** Role of bioinformatics in the development of personalized medicine

| |
|---|
| **Role of bioinformatics in molecular diagnostics as applied to personalized medicine** |
| Analysis and classification of gene expression profiles |
| Analysis of single nucleotide polymorphisms |
| Computational diagnostics |
| Diagnosis of subtype of a disease to select the probability of success of optimal treatment |
| Genetic screening |
| **Role of bioinformatics in pharmacogenomics** |
| Genotyping for stratification of clinical trials |
| Selection of targets in pharmacogenomics-based drug discovery |
| Use of pharmacogenomic data to develop rational therapies |
| **Role of bioinformatics in pharmacogenetics** |
| Analyzing the role of polymorphisms in interindividual variations in drug response |
| Computational tools for predicting drug metabolism, toxicity and efficacy |
| Integration of pharmacogenetic data with clinical outcomes to facilitate diagnosis |
| Link pharmacogenetic data to literature on adverse reactions and drug-drug interactions |
| **Role of bioinformatics in pharmacoproteomics** |
| Analysis of data from protein microarrays |
| Measurement of protein expression |
| Search engines for proteomic databases |
| Biosimulation and machine learning techniques for developing personalized medicine |
| **Applications in organization of personalized medicine** |
| Personalized prognosis of disease |
| Linking patient-specific and knowledge-based information |
| Linking patient medical records and genetic information |
| Monitoring of health status by digital devices |

predict clinical outcome based on the chosen features. Computational diagnostics includes identification of novel, molecularly defined entities of a disease. For many clinical decision problems where a large number of features are used to monitor a disease, neural networks and other machine-learning approaches can help to manage the situation.

The impact of having the human sequence and personalized digital images in hand has also created tremendous demands of developing powerful supercomputing, statistical learning and artificial intelligence approaches to handle the massive bioinformatics and personalized healthcare data, which will obviously have a profound effect on how biomedical research will be conducted toward the improvement of human health and prolonging of human life in the future. The International Society of Intelligent Biological Medicine (http://www.isibm.org) touches future bioinformatics and personalized medicine throughout current efforts in promoting the research, education and awareness of the upcoming integrated inter/multidisciplinary field (Yang et al. 2008).

Wireless non-invasive biosensors are in development for monitoring of all vital signs including continuous blood pressure, heart rhythm, oximetry, respiratory rate, and temperature. A subcutaneous sensor can provide a highly accurate reading of glucose every 5 min for continuous glucose monitoring of diabetics. A cell-phone-sized device can be used to acquire high-resolution 2D echocardiography and color flow. Consumers will soon learn how to acquire their own echocardiograms, fetal ultrasounds, or breast ultrasounds, and transmit the images for their physicians for real-time interpretation. Along with genomic information, digital technologies will facilitate the practice of personalized medicine.

### *Exploration of Disease-Gene Relationship*

LARaLink 2.0 (Loci Analysis for Rearrangement Link) is an enabling web technology that permits the rapid retrieval of clinical cytogenetic and molecular data. New data mining capabilities have been incorporated into version 2.0, building upon LARaLink 1.0, to extend the utility of the system for applications in both the clinical and basic sciences. These include access to the Chromosomal Variation in Man database and the GEO database. Together these new resources enhance the user's ability to associate genotype with phenotype to identify potential gene candidates. Unlimited access for researchers exploring disease-gene relationships and for clinicians extending practice in patient care is available online (LARaLink.bioinformatics.wayne.edu:8080/unigene).

### *Biosimulation Techniques for Developing Personalized Medicine*

An example of this is REFS™ (Reverse Engineering and Forward Simulation) technology used by Gene Network Sciences Inc in pharmaceutical and clinical settings to rapidly turn combinations of genetic, genomic, and clinical measurements into mod-

els of disease progression and drug response. These computer-assembled models are then queried rapidly through billions of in silico experiments (Forward Simulation) to discover the highest-impact molecular targets for the disease being studied and the corresponding efficacy and toxicity biomarkers related to specific drug treatments. These findings are then tested in both the laboratory and the clinic, enabling a faster, more-focused drug discovery and development process. Advantages of REFS™ are:

- Identification of the molecular causes of disease, rather than those factors that are merely correlative, thereby identifying novel, first-in-class targets, and efficacy and toxicity biomarkers
- Improvement of the quality of preclinical drug candidates and the probability of a drug candidate's phase I/II and subsequent successes through improved understanding of the mechanisms of efficacy and toxicity.
- Guiding the design of clinical trials by stratifying the patient population to maximize the response rate of patients in clinical trials.
- Improving treatment decisions by physicians for patients with complex diseases.

REFS™ is being applied in a research collaboration with M.D. Anderson Cancer Center aimed at the rapid translation of DNA sequence and clinical data from patients with glioblastoma multiforme, into breakthrough discoveries leading to drugs and diagnostics for personalized management of patients.

## *Health Information Management*

Bioinformatics can also help in health care information management. Personalized medicine involves linking two types of information: patient-specific and knowledge-based. Personal information is documented in patient records. Some personal medical documents, which are already in use to various extents in different countries, include the personal emergency card, the mother-child record, and the vaccination certificate. A more valuable but under-used source of personal medical information is the data stored in the electronic health records, which needs to be used universally for facilitating the development of personalized medicine.

## *Electronic Health Records*

Electronic health records (EHRs) are important for improving healthcare and for widening the scope of personalized medicine as they can be shared online by different physicians and hospitals. They can improve the quality and safety of patient care by reducing errors in prescriptions. EHRs facilitate clinical trials and collection of adverse drug events data. In the aftermath of Hurricane Katrina in New Orleans in 2005, government and private health care officials were rushing to build an electronic database of prescription drug records for hundreds of thousands of people who lost their records in the storm. This tragic happening powerfully demonstrated the need for EHRs.

Major healthcare organizations like Kaiser Permanente Group, the Mayo Clinic and other centers in the US have spent billions of dollars to convert to EHRs. Medicare and some employers are paying incentives to medical providers that can achieve better efficiency and patient care through improved information management. Smaller medical practices, where majority of the US patients are treated, lagged behind in adoption of EHRs because of the high initial costs involved and the need for support and training. As of 2008, only 13 % of US physicians had basic EHR system and 4 % reported having an extensive, fully functional EHR system (DesRoches et al. 2008). Another survey found that only 1.5 % of US hospitals had a comprehensive EHR system (i.e. present in all clinical units), and an additional 7.6 % had a basic system (i.e. present in at least one clinical unit). Computerized provider-order entry for medications had been implemented in only 17 % of hospitals (Jha et al. 2009). Larger hospitals, those located in urban areas, and teaching hospitals are more likely to have electronic-records systems. The adoption of HER has increased since 2008. Taconic Health Information Network in New York State introduced an affordable and practical system for computerization of patient records in small medical practices. This led to reduction of medication errors and redundant procedures while improving diagnostic accuracy and facilitating electronic prescribing.

In 2009, the US Congress provided the health care community with a transformational opportunity to break through the barriers to progress. The Health Information Technology for Economic and Clinical Health Act (HITECH) authorized incentive payments through Medicare and Medicaid to clinicians and hospitals when they use EHRs privately and securely to achieve specified improvements in care delivery. Through HITECH, the US Federal Government has committed unprecedented resources to supporting the adoption and use of EHRs. The DHHS is committed to the support, collaboration, and ongoing learning that will mark the progress toward electronically connected, information-driven medical care (Blumenthal and Tavenner 2010).

Janssen Diagnostics' AVIGA™, a subscription EHR that harnesses and transforms data into knowledge with its analytical power, boosts the health information system. Along with AVIGA REPORTER™, a reporting and research tool designed for use with another EHR, clinicians and healthcare professionals can assimilate the information needed to make timely, informed treatment decisions for their patients.

**Cost of EHR and Savings on Healthcare Expenses in the US**

RAND Corporation researchers projected in 2005 that rapid adoption of EHR could save the US > $81 billion annually. Seven years later adoption of HER had failed to produce the hoped-for savings in health care costs and has mixed results, at best, in improving efficiency and patient care, according to a new analysis by RAND (Kellermann and Jones 2013). Health care expenditures in the US have grown by $800 billion. The disappointing performance of EHR can be largely attributed to several factors: sluggish adoption of EHR systems, coupled with the choice of systems that are neither interoperable nor easy to use; and the failure of health care

providers and institutions to reengineer care processes to reap the full benefits of these systems. The original promise of EHR can be met only if the systems are redesigned to address these flaws by creating more-standardized systems that are easier to use, are truly interoperable, and afford patients more access to and control over their health data. Providers must do their part by reengineering care processes to take full advantage of efficiencies offered by EHRs, in the context of redesigned payment models that favor value over volume.

### EHRs and Genome-Wide Studies

The National Human Genome Research Institute (NHGRI) is funding the development of methods and procedures for using EHRs in genome-wide studies that rely on biorepositories. NHGRI has funded groups affiliated with existing biorepositories to develop methods and procedures for genome-wide studies in participants with phenotypes and environmental exposures defined by EHRs, with the intent of widespread sharing of the resulting individual genotype-phenotype data. The program will consider and address issues of consent and consultation connected to biorepository-based research, genome-wide technologies, and data sharing. The institute will support studies such as harmonizing phenotypes, developing data-capture methods and analytic strategies, assessing data quality and potential biases, and evaluating or improving consent or data protection processes.

### Linking Patient Medical Records and Genetic Information

IBM's Genomic Messaging System (GMS) provides a basic computer language that can be inserted into DNA sequences to bridge the gap between patient medical records and genetic information. GMS was originally developed as a tool for assembling clinical genomic records of individual and collective patients, and was then generalized to become a flexible workflow component that will link clinical records to a variety of computational biology research tools, for research and ultimately for a more personalized, focused, and preventative healthcare system. Prominent among the applications linked are protein science applications, including the rapid automated modeling of patient proteins with their individual structural polymorphisms. In an initial study, GMS formed the basis of a fully automated system for modeling patient proteins with structural polymorphisms as a basis for drug selection and ultimately design on an individual patient basis.

Genetic data obtained by use of microarrays needs to be integrated with existing medical records and then be made readily accessible to the practicing physician in a standardized format such that enables information from one patient can be readily compared to another. Affymetrix is collaborating with IBM to facilitate the integration of genomic research and patient clinical data from several databases into a centrally organized format. The combination of standard medical information with microarray genetic data will then be cross-referenced against the databases enabling

genetic clinical research to be translated into clinical application. A US Department of Health and Human Services team is focused on integrating genomic data with medical records to facilitate the development of personalized medicine. In 2009, Aurora Health Care (Milwaukee, WI), which has close to two million patients, unveiled its automated biorepository at St. Luke Medical Center. It has large-scale data-sharing plans to integrate health records with omics data.

Optimal clinical use of genetic test results and molecularly-targeted therapies present important challenges in patient management, which can be effectively addressed by using electronic clinical decision support technologies. A working group of the American Health Information Community has conducted assessment of needs for clinical decision support in electronic health record systems to support personalized medical practices. An action plan was suggested for government, researchers and research institutions, developers of electronic information tools (including clinical guidelines, and quality measures), and standards development organizations to meet the needs for personalized approaches to medical practice. An excellent publication has discussed the activities of stakeholder organizations to identify and coordinate needs and opportunities for clinical decision support tools to enable personalized medicine (Downing et al. 2009).

### Management of Personal Genomic Data

Patient genomic data would be important for clinical decision making in a personalized medical system. The management of such sizeable, yet fine-grained, data in compliance with privacy laws and best practices presents significant security and scalability challenges. GenePING, an extension to the PING personal health record system, is the first personal health record management system to support the efficient and secure storage and sharing of large genomic datasets (Adida and Kohane 2006). The design and implementation of GenePING has been published. It supports secure storage of large, genome-sized datasets, as well as efficient sharing and retrieval of individual datapoints (e.g. SNPs, rare mutations, gene expression levels). Even with full access to the raw GenePING storage, it would be difficult for a hacker to access any stored genomic datapoint on any single patient. Given a large-enough number of patient records, an attacker cannot discover which data corresponds to which patient, or even the size of a given patient's record. The computational overhead of GenePING's security features is a small constant, making the system usable, even in emergency care, on today's hardware.

### Use of EHRs for Improving Safety of New Medicines

Genetic variations influence susceptibility to adverse drug reaction (ADRs), and predictive genetic tests have been developed for a limited number of ADRs. The identification of patients with ADRs, obtaining samples for genetic analysis and rigorous evaluation of clinical test effectiveness are significant challenges for

development of predictive genetic tests. Using the example of serious drug-induced liver injury, a study has shown how a database of routinely collected EHRs can be used to overcome these barriers by facilitating rapid recruitment to genome-wide association studies and supporting efficient randomized controlled trials of predictive genetic test effectiveness (Wing et al. 2014).

### Use of EHRs for Genetic Research

EHRs are a potential source of longitudinal clinical data for research. The Electronic Medical Records and Genomics Network (eMERGE), including its partners at Northwestern, Mayo Clinic, Vanderbilt University, Marshfield Clinic Research Foundation, and Group Health, has investigated whether data captured through routine clinical care using EHRs can be used to identify disease phenotypes with sufficient positive and negative predictive values for use in genome-wide association studies. Using data from 5 different sets of EHRs – type 2 diabetes, dementia, peripheral arterial disease, cataracts, and cardiac arrhythmia – the investigators have identified five disease phenotypes with positive predictive values ranging from 73 % to 98 % and negative predictive values ranging from 98 % to 100 % (Kho et al. 2011). Most EHRs captured key information (diagnoses, medications, laboratory tests) used to define phenotypes in a structured format. The authors showed that natural language processing is an important tool for improving case identification rates. Efforts and incentives to increase the implementation of interoperable EHRs will markedly improve the availability of clinical data for genomics research. Researchers studying the underlying genetic causes for human diseases can dramatically cut their costs and save time by mining data about real patients found in EHRs, instead of recruiting and sorting participants as they look for common genetic variants. As the cost of genome sequencing is dropping, it should eventually be possible to include patients' genomes in their medical records, providing a valuable source of information for disease researchers. The larger the studies, the better they could be at detecting rare effects of genes and providing more detail about the genetic sequences that lead to diseases. Drawbacks of EHR-based research are: (1) lack of uniformity as the EHRs used different software; and (2) EHRs did a poor job of capturing different factors such as race and ethnicity, smoking status, and family history.

Although the use of EHRs is becoming well established in some health care systems in the US, such as those of Kaiser Permanente and the Geisinger Health System, the integration of genetic information is lagging behind as there are 10 different EHR systems. According to the results of a survey of health care professionals, only 4 % of the respondents reported that their EHR system provided any decision support on the basis of the results of genetic tests, and the vast majority reported that their EHR supplier did not provide the type of support they needed for the interpretation of genetic information (Scheuner et al. 2009).

EHRs could provide a platform for the integration of genetic information into clinical practice by guiding clinicians about when to order a genetic test, how to

document and interpret the results, how to apply the information for treatment decisions and prevention screening, and when to refer patients for genetic counseling. Such automated guidance is vital for both health care workers and their patients. EHRs also facilitate research with large cohorts, a factor that is especially valuable for prospective studies of genetic and environmental effects on health in which the linking of phenotype to genotype is essential. Translational research is converting new knowledge gained from EHRs into insights about prevention and treatment of diseases. The challenge is to ensure that innovation in research and medicine is equaled by policies that foster science while protecting and respecting research participants and patients (Hudson 2011).

**Use of EHRs for Personalized Drug Discovery and Development**

Although the application of EHRs is beneficial for patient care in a hospital or clinical setting, it can also aid drug discovery. EHR databases are already being used in the pharmaceutical industry for market research, pharmacovigilance, clinical biomarker validation and drug safety evaluation. Because EHRs provide observational data for a large population over long periods of time, it is possible to utilize them for a better understanding of how drugs affect patients through changes in diagnosis, disease progression and laboratory measurements. Specific applications of EHRs in a drug discovery include finding novel relationships between diseases, re-evaluating drug usage and discovering phenotype-genotype associations (Yao et al. 2011). In the near future EHR systems and related databases will have a significant impact on how we discover and develop safe and efficacious medicines.

## *Personalized Prognosis of Disease*

Computational and Applied Genomics Program of the Duke University (Durham, NC) has developed a comprehensive modeling approach to combining genomic and clinical data for personalized prediction in disease outcome studies. This integrated clinicogenomic modeling framework is based on statistical classification tree models that evaluate the contributions of multiple forms of data, both clinical and genomic, to define interactions of multiple risk factors that associate with the clinical outcome and derive predictions customized to the individual patient level. Gene expression data from DNA microarrays is represented by multiple, summary measures termed metagenes; each metagene characterizes the dominant common expression pattern within a cluster of genes. A case study of primary breast cancer recurrence demonstrates that models using multiple metagenes, combined with traditional clinical risk factors, improve prediction accuracy at the individual patient level, delivering predictions more accurate than those made by using a single genomic predictor or clinical data alone. The analysis also highlights issues of communicating uncertainty in prediction and identifies combinations of clinical and genomic risk factors playing predictive roles. Implicated metagenes identify gene

subsets with the potential to aid biological interpretation. This framework will extend to incorporate any form of data, including emerging forms of genomic data, and facilitate development of personalized prognosis.

## Global Scope of Personalized Medicine

Development of personalized medicine needs to be considered against the background of current healthcare trends, which vary from one country to another. Basic healthcare depends on the economic resources, political systems, healthcare organization, government support and allocations of finances. There are differences in healthcare standards between the developing and the developed countries. Personalized medicine will be initially introduced in the Western developed countries. US is likely to be the first country to introduce personalized medicine on a large scale and some countries in the EU will follow.

### *Global Alliance for Genomics and Health*

The Global Alliance for Genomics and Health (http://genomicsandhealth.org/) is an independent, non-governmental alliance, made up of hundreds of world-leading organizations and individuals from across the world, who are dedicated to improving human health by maximizing the potential of genomic medicine through effective and responsible data sharing. The promise of genomic data to revolutionize biology and medicine depends critically on our ability to make comparisons across millions of human genome sequences, but this requires coordination across organizations, methods, diseases, and even countries. The members of the Global Alliance are working together to create interoperable approaches and catalyze initiatives to help unlock the potential of genomic data.

Since its formation in 2013, the Alliance has grown to 218 member organizations in 27 countries and is leading the way to enable genomic as well as clinical data sharing. Its Working Groups are producing high-impact deliverables to ensure that responsible sharing is possible, such as developing a Framework for Data Sharing to guide governance and research and a Genomics API to allow for the interoperable exchange of data. The Working Groups are also catalyzing key collaborative projects that aim to share real-world data.

### *Personalized Medicine in Canada*

Canada has one of the best healthcare systems in the world with the widest coverage of its population. Considerable advances have taken place in biotechnology for healthcare in recent years and these are being translated into clinical applications. There are a number of examples of personalized programs and some are briefly described here.

**Personalized Medicine at Ontario Institute for Cancer Research**

In 2010, the Ontario Institute for Cancer Research (OICR) announced that it will expand efforts to translate its research over the next 5 years, in part through a greater focus on personalized medicine and building partnerships with industry and other research institutions, according to its Strategic Plan 2010–2015 (http://www.oicr.on.ca/). OICR will join with partners in academia, industry, healthcare organizations, and government on four priorities it identified for its translation effort: (1) adopting more of a personalized medicine approach toward fighting cancer; (2) developing solutions to clinical issues that could benefit patients in the next 5 years; (3) digitizing and improving interpretation of cancer data; and (4) accelerating OICR's Patents to Products Program, which encourages Ontario-based companies to develop, use, and commercialize products based on the institute's research. The institute will also work to generate commercialization opportunities within the Canadian province through a new partnership it has formed with the Ontario Medical Advisory Secretariat (MAS), a division of the Ministry of Health and Long-Term Care (MoHLTC); and Cancer Care Ontario, the provincial agency overseeing cancer services, including the nearly C$700 million ($692 million) public healthcare dollars to cancer care providers.

The initial goals of OICR are to establish prognostic and predictive genetic tests in cancers that guide the proper and effective use of cancer therapies. The program will monitor efficacy and produce full economic analyses. Over time, other modalities of personalized medicine will be dealt with. Its partnership with CCO and MoHLTC will focus on molecular tests used to guide the use of targeted therapies, such as the Her2 test for trastuzumab and K-RAS mutation testing for EGFR inhibitors. The tests will also help the partners decide if characteristics of tumors warrant more conventional cancer treatment, the report stated. The partnership may evolve to enable mechanisms that would measure the effects of treatments at earlier stages, and/or allow treatment optimization of targeted therapies at all stages (prior, during, or after). The partnership will build support for startups focusing on developing personalized medicine products – including pharmacogenomics, target identification/drug development, diagnostic, and imaging – and work with regulatory agencies to streamline reviews for new therapies and diagnostic and prognostic tests. OICR also committed itself to addressing five clinical challenges over the next 5 years:

1. Identifying targets and new therapies through its large-scale genomic analyses of pancreatic cancer.
2. Discovering urine, serum, imaging, and pathological biomarkers that predict prostate cancer, with the goal of preventing over-diagnosis of patients. OICR will team up with Prostate Cancer Canada and Cancer Research UK to generate comprehensive genome datasets from indolent and aggressive tumors, from which new candidate biomarkers would be identified.
3. Developing imaging and pathological biomarkers that predict the risk of breast cancer.

4. Creating programs to increase the number of patients screened for colorectal cancer and increase participation in Ontario's 5-year ColonCancerCheck initiative to establish a colorectal cancer screening program.
5. Partnering with other Canadian agencies seeking to create a national program to improve quality of life for young cancer survivors. OICR could help by examining the long-term effects of cancer drugs.

To promote digitizing and improving interpretation of cancer data, OICR will promote partnerships between academic teams and Ontario-based companies; stoke further discussions among cancer researchers, computer scientists, healthcare providers, and medical IT companies; develop a comprehensive tech platform for storing, exchanging, and analyzing datasets obtained from human subjects; and provide grants and other incentives to research groups able to create new algorithms, data storage solutions, and clinical tools for data visualization interfaces and decision support tools.

The institute also said it will increase the size and scope of its commercialization program over the next 5 years, in part by working to attract industry partners and private investors to companies they and the institute will help create. OICR will also pursue large-scale collaborations with multinational therapeutics and diagnostics companies interested in a provincial presence, and create new networks of investors and business partners, drawing on existing programs like the MaRS Discovery District in Toronto and Innovation Accelerator Fund, as well as Ontario-based clinical trials groups.

In the strategic plan, OICR offered 5-year direction for several existing programs involving the institute. The Cancer Genomics Program, for example, will expand its scope to a large number of patients and several types of tumors through genomic studies of tumors collected from other programs, such as High Impact Clinical Trials, with the goal of developing future personalized medicine strategies for several common and rare cancers. The Cancer Stem Cell Program will identify new targets for discovery of specific anti-CSC agents designed to eradicate the stem cells of various tumors. Initiatives over the next 5 years, according to OICR, will allow it to use and further develop its technology platforms – imaging pipeline, transformative pathology, genome technologies, medicinal chemistry, and informatics and biocomputing.

OICR is also conducting a cancer tumor resequencing and analysis project that seeks to serve as a framework for how sequencing may be used in the clinic to develop tailored cancer therapies (Dancey et al. 2012). The study is based in part on sequencing that currently is being conducted by the International Cancer Genome Consortium, which has shown that some mutations associated with one type of cancer, such as the BRAF mutation, have been observed in other types of cancer. The publication covers a number of the issues related to use of genome sequencing in cancer trials, including tissue requirements, patient recruitment and informed consent, data sharing, and the implications of such projects and data on drug development, regulatory agencies, patients, providers, and others. It offers proposals and shares practices based on lessons OICR researchers have learned in trying to incorporate sequencing in cancer care and clinical trials. Findings of this study suggest that cancer diagnosis should

involve an in-depth analysis of a tumor's mutation for many different types of cancer, regardless of where the tumor originated. OICR's recent sequencing projects have been building up the foundation for this large-scale clinical sequencing project. OICR has already started clinical resequencing of patients with metastatic disease, resequencing a large number of target genes with the Pacific Biosciences system. These projects have been testing the feasibility of moving to a large-scale study. One of OICR's central goals with this project is to use sequencing to test for a range of mutations in tumors, instead of just one type that has already been shown to have a meaningful association with treatment or outcome. The aim of this effort is to conduct molecular profiling by sequencing, rather than genotyping, so that patients can be moved to the appropriate clinical trials. The results of such trials would help to determine the treatments that would be given to individual patients.

### Personalized Medicine Partnership for Cancer in Quebec

In February 2013, The Government of Québec announced a $10 million investment in the Personalized Medicine Partnership for Cancer (PMPC). The public-private partnership will be focused on establishing an integrated approach for the development and implementation of clinical biomarkers and other personalized healthcare solutions to improve the outcome and cost efficiency of healthcare services provided to cancer patients in the province of Québec and abroad. The investment, to be disbursed over a 4 year period, will be supplemented with $11.1 million of funding from the private sector partners, for a total project value of $21.1 million. PMPC will be under the leadership of Caprion Proteome, a company specializing in the discovery and development of protein-based diagnostic biomarkers. Other partners will include the Québec Clinical Research Organization in Cancer, a multidisciplinary network of clinicians, academic scientists and other members of the medical community involved in clinical and translational cancer research, as well as private partners Oncozyme Pharma, Pfizer Canada, Sanofi Canada and TELUS Health, a healthcare service provider. As part of the projects supported through this partnership, state-of-the-art genomic, proteomic, bioinformatic and information technology platforms will be implemented to develop and deploy novel biomarkers and targeted therapeutic strategies in the healthcare system for the treatment of lung, colon and breast cancers. This partnership will integrate advanced technology platforms with clinical research to accelerate the development and clinical deployment of novel personalized healthcare solutions.

### Quebec Center of Excellence in Personalized Medicine

In 2008, Montreal Heart Institute and Génome Québec formed the Center of Excellence in Personalized Medicine, which will be funded with more than $22 million in investments from government and commercial entities over 5 years.

Canada's Centers of Excellence for Commercialization and Research program will provide $13.8 million of the total funding, with the remainder coming from private and public partners including the ministère du Développement économique, de l'Innovation et de l'Exportation of Québec. The goal of the new center is to develop approaches and methods that will optimize treatment and ensure their rapid and productive transition from the research stage to use in clinical practice. The Montreal Heart Institute will house the new center, which was developed in collaboration with pharmaceutical and biotech companies.

## *Personalized Medicine in the EU*

There is a tremendous variation in the healthcare systems within the EU but there are some emerging patterns. A 2004 EU consumer survey showed that:

- 1/3 of consumers are willing to spend private money for healthcare
- 2/3 are ready to cross borders to access better care
- 3/4 ask for better access to information

All EU systems are converging around common denominators including: more powerful patient organizations, stricter cost control measures, enhanced use of informatics. Patient bodies are part of decision-making in most EU systems, even the EMEA, unlike the FDA in the US. There is an increasing impact of EU regulatory bodies on national healthcare systems. As was previously true of the managed care movement, healthcare systems in Europe would likely learn from the CDHC movement in the US and integrate lessons and tools from that movement into the frameworks of their given systems.

These trends in healthcare would be favorable for the development of personalized medicine. The following European countries appear likely to develop personalized medicine ahead of others: UK, Sweden, Spain and Germany. The current situation in the UK is more favorable to the development of personalized medicine than other EU countries.

### European Personalized Medicine Diagnostics Association

European Personalized Medicine Diagnostics Association (EPEMED) is a nonprofit organization in Europe that, similar to the Personalized Medicine Coalition in the US that will focus on promoting and harmonizing personalized medicine and implementing high value diagnostics across the continent. Made up of biotechnology firms, academic and institutional researchers, small and large businesses, and patient advocacy groups, announced its board of directors this week. Included on that board are executives from QIAGEN Marseille, Genzyme, the Personalized Medicine Coalition, BioMerieux, Theranostics, and Novartis Molecular Diagnostics.

EPEMED's central goals are to move personalized medicine forward throughout Europe through targeted education, idea-sharing, and business models, and to promote harmonization in Europe's approach to personalized medicine. The group also plans to create joint programs with other international personalized medicine organizations, and to offer opinions on policies related to the field. EPEMED expects that the coming EPEMED's responsibility to make sure that these innovations will be made available to European patients and as a result, to make Europe an attractive place for innovations, financial and industrial investments in the area of personalized medicine diagnostics.

### UK National Health Service and Medical Genetics

UK genetic services are among the most highly developed in Europe, having evolved from academic departments into regional centers. Regional genetic centers are multidisciplinary, with clinical and laboratory services united or working closely together. Each center includes specialist clinics and clinics in district hospitals and community facilities. Outreach staff from some centers may visit families at home. Genetic services help families with the risk of a genetic disorder to live as normally as possible. After a consultation and investigations patients are given information about the condition in their family, their risk of developing or transmitting the condition, and the options for dealing with it (genetic counseling).

The UK government awarded a package of £30 m ($42 m) in 2001 for measures to help bring the genetics revolution into everyday medical practice. A White Paper titled " Our Inheritance, Our future: realizing the potential of genetics in the NHS" was published in 2003 (www.tso.co.uk/bookshop). This document depicted the Government's strategy for maximizing the potential of genetics in NHS so that all patients can benefit from new genetic advances in disease prevention, diagnosis and treatment. Under the UK government plan, the number of consultants specializing in genetics will nearly double to 140 by 2006. Support staff and genetic counselors will also double in number to about 450. Research and development in pharmacogenetics will be supported. The numbers of patients being seen by specialist genetic services will increase by about 80 % to 120,000 a year, and the wait to see a specialist is set to fall from about a year to 3 months. The White Paper generally avoids the area of widespread population screening except in flagging up the antenatal and the newborn screening programs. The possibility of genetically profiling every newborn child to guide lifetime decisions has been considered. Overall, the White paper represents an important milestone in the development of a rational policy for the application of genetic science in healthcare services in the UK.

In 2009, the NHS invested £4.5 million ($7.4 million) in a new pilot program to prepare its physicians for the changes that personalized and genomic medicine will bring to the healthcare field. The goal of the new program, funded by the Department of Health under the UK Modernizing Scientific Careers program, is to provide enhanced training in genetic technologies and clinical applications for healthcare

scientists working in laboratory genetics. Starting in 2009, the program has funded 24 pilot training posts for 12 trainee Healthcare Science Practitioners and 12 Healthcare Scientists in Genetics. These trainees are being based in a number of NHS genetics departments throughout England, and they will meet for national training events. The pilot will have four components and goals including establishing a national School of Genetics in the West Midlands; modernizing the genetics curricula to respond to breakthrough scientific advances and their applications for patients and the public; responding to future workforce needs to keep up with discoveries from the last decade about how to diagnose and predict disease; informing other healthcare science training programs that began in 2010 and were implemented in 2012. This initiative by the NHS makes UK a promising place to introduce personalized medicine.

**Personalized Medicine in Germany**

Next to UK, Germany has the most activity for the development of personalized medicine in Europe. German academic institutions have been active in genomic research for several years. There are several programs in pharmacogenomics and pharmacogenetics. Germany has more medical diagnostic and personalized medicine companies than any other country in the EU with the exception of UK. Government support of personalized medicine is exemplified by the grants given to promote research and development in personalized medicine. In 2010, Government of Nordrhein-Westfalen gave grants worth €25 million ($35 million) to 9 research consortia for personalized medicine. The state will gain €1.3 billion by 2013 from EFRE-Fund of the EU. Beneficiaries of these grants will be networks of universities, research institutes, and biotechnology companies. These include Ruhr-University Bochum, University Klinic Essen, University of Cologne, University of Bielefeld, Association for Advancement of Analytical Sciences, Lead Discovery Center GmbH, Life & Brain GmbH, and Miltenyi Biotec. Research topics will include new techniques of diagnosis, effective therapies to improve patient care and search for biomarkers of diseases such as cancer, liver disease, Alzheimer disease and arteriosclerosis. Cell-based therapies and stem cell research are also included.

The German Ministry for Education and Research ran a contest for excellence and a cluster of personalized companies, Bio$^{M}$, in Munich won a prize of €40 million as research grants. This prize will be matched by donations of equal amounts from the industry and the state government of Bavaria. The cluster of companies has set up 40 collaborations and seven projects to bridge the gap between the industry and the academia. The most important proposal is formation of a center, M4, for personalized medicine where companies could carry out phase I and phase II clinical trials with an aim to gain approval for 50 products within the next decade. The M4 center will also house a tissue bank, where local companies will have access to blood and tissue samples for research.

## *Personalized Medicine in Israel*

The Israel National Center for Personalized Medicine (INCPM), located at the Weizmann Institute of Science, represents a unique opportunity to build on the foundation of excellence in genomics and bioinformatics in Israel. Its multidisciplinary, multi-institutional teams will conduct collaborative research that will lead to major discoveries in the genetic and molecular basis of disease and translate them into clinical practice. The institutes involved are:

Crown Institute for Genomics combines the innovation of the Weizmann Institute with cutting edge industry standards and state-of-the-art DNA and RNA NGS technologies. It aims to become more than a service provider; its goal is to combine sequencing services with research and development focused at overcoming the bottlenecks and limitations associated with assay development, automation, and data analysis. Taken together, these capabilities will help turn the INCPM into a unique single entity capable of partnering with researchers on all levels of study, boosting Israel's position in this rapidly developing field of research.

de Botton Institute for Protein Profiling (Proteomics) is ultimately geared toward exhaustively characterizing all proteins in the human body, in health and disease. A 'bottom-up' approach is used where proteins are extracted from the biological samples, subjected to enzymatic digestion followed by liquid chromatography – mass spectrometric analysis. Post-acquisition, the protein identity and quantity is reconstructed using the latest bioinformatics.

Maurice and Vivienne Wohl Institute for Drug Discovery. The HTS Unit will act as a nationally accessible central resource, open to all qualified Israeli researchers at universities, research institutes and biopharmaceutical companies, to provide screening in support of their needs to identify research tools to support biomedical discovery and therapeutic and prophylactic molecules.

Ilana and Pascal Mantoux Institute for Bioinformatics will be providing the computing power and environment required for analysis. High performance cluster and storage array hosting various analysis and visualization tools will be made available to all collaborating scientists.

## *Personalized Medicine in the Developing Countries*

Poor persons in the developing countries and even in the developed countries of the West have not benefited from some of the advances in modern medicine. Would personalized medicine be applied to the economically deprived? It is unlikely that some of the basic problems of medical care for the poor will be resolved during the next decade to consider personalizing the medical care. If patients in Africa have difficulties in getting anti-HIV drugs because of the high cost, genotyping for personalizing care and overcoming drug resistance is a secondary consideration. A concern has been expressed that as pre-emptive treatments become available, the rich in the developing and the developed nations will consume these to avoid

genetically predisposing risks without having to change their lifestyle. Rather than worrying about such theoretical concerns, the emphasis should be on sharing genomic information with developing countries and using it to develop cost-effective population-based treatment for endemic diseases in the developing countries such as malaria and tuberculosis. Personalized medicine may eventually prove to be more economical than conventional medicine. One reason for investigating personalized medicine further in the developing countries would be ethnic variations in drug response based on pharmacogenetics as currently available pharmacogenetic data do not comprehensively explain drug response variation within the human populations. One of the many reasons the solutions are incomplete is that they are focused on Western patient donors. The genetic causes for variable drug response are heterogeneous among the various nations of the world, and a classification/diagnostic kit that works very well for Caucasians may work poorly for individuals of Asian descent. To generate complete, broadly useful and sensitive drug-patient classification kits, population studies of international representation are required.

Southeast Asian populations and ethnic subgroups have been poorly represented in genomics research and product development efforts. The vast majority of pharmacogenomics research is conducted in North America and Europe primarily because of the difficulties in obtaining specimens from countries such as Malaysia, Indonesia and many other Southeastern Asian countries. To remedy this situation, a subsidiary was established by DNAPrint Genomics in collaboration with a Malaysian biotechnology company – DNAPRO SDN BHD (Kuala Lumpur, Malaysia), DNAPrint. The new company has secured access to a broad range of specimens that allow for the development of pharmacogenomics classification products for this specific population of Southeastern Asian descent. The results would be available for application to healthcare of nearly 3.5 billion people worldwide who are of Southeast Asian descent.

## Advantages of Personalized Medicine

Advantages of personalized medicine for those involved are tabulated as follows: the biopharmaceutical industry (Table 20.4), the patients (Table 20.5), the physicians (Table 20.6), and the healthcare providers (Table 20.7).

**Table 20.4** Advantages of personalized medicine for the biopharmaceutical industry

| |
|---|
| Reduced costs of drug development |
| Reduced time for drug development |
| Monopoly in a specified segment of the market |
| Increase in discovery of new drugs |
| Increased revenues from combination of diagnostics packaged with therapeutic products |
| Reduction of the need for black-box warnings |
| Rescue of failed drugs by matching them to patients for whom they are safe and effective |

**Table 20.5** Advantages of personalized medicine for the patients

| |
|---|
| Participation in decision-making about choice of treatment |
| Effective and specific therapies |
| Less risk of adverse effects |
| No time lost in trial and error with ineffective drugs |
| Lower cost of treatment |
| Facilitates personalized preventive healthcare |
| Improvement of quality of life |



**Table 20.6** Advantages of personalized medicine for the physicians

| |
|---|
| Avoidance of trial and error approach in selection of drugs |
| Rational therapeutic decisions based on pathomechanism of disease |
| Diagnostic guidance to treatment incorporated in personalized approach |
| Less complications of treatment and adverse effects of drugs |
| Increased professional satisfaction |
| Advances in medicine and translation of new biotechnologies into clinical practice |



**Table 20.7** Advantages of personalized medicine for the healthcare providers

| |
|---|
| Saving healthcare costs in management of chronic diseases |
| Reduced adverse effects and complications of treatment that will reduce cost of care |
| Companion diagnostics will prevent misuse of new expensive biological therapies |
| Improved patient compliance |



## Limitations of Personalized Medicine

Limitations of personalized medicine are shown in Table 20.8.

One of the limitations of pharmacogenomics-based medicine is that there is a lot more to drug response than genes. Drug treatment outcome represents a complex phenotype, encoded by dozens, if not hundreds, of genes, and affected by many environmental factors; therefore, we will almost always see a gradient of response. Diet, general health, and drug-drug interactions are just some of the factors that alter a drug's performance in a given patient. The genome is not going to give us all the answers, just some of the answers. The other factors will need to be studied as well.

The laudable, longer term objective of personalized medicine cannot be fulfilled however, until one more element of diagnostic testing becomes feasible by the creation of reliable methods to predict how an individual's unique genetic status may predispose him/her to the development of future illness. The development of disease predisposition risk diagnostic tests that map the probability that an individual will succumb to one or more of the complex late-onset, multigenic, non-mendelian diseases that

**Table 20.8** Limitations of personalized medicine

| |
|---|
| Factors other than genes also affect response to drugs |
| Not all the treatments can be personalized |
| Limited support from politicians or governments |
| Lack of knowledge of personalized medicine among physicians |
| Ethical, legal and social problems need to be addressed |
| Approval of new biomarkers from regulatory agencies is difficult |
| Shortage of bioinformatic manpower needed for management of huge amounts of data |
| Technologies required for implementation of personalized medicine still need refinement |
| Routine genetic testing revealing clinically non-relevant information – Incidentalome |



account for most patient morbidity and mortality is the most futuristic and technically the most complicated, element of the emerging diagnostic universe.

New genome-scale screening tests may lead to a phenomenon in which multiple abnormal genomic findings are incidentally discovered, analogous to the "incidentalomas" that are often discovered in radiological studies. The "Incidentalome" in radiology has some benefits resulting from discovery of unexpected potentially life-threatening conditions that can be treated prior to clinical manifestations. However, the incidentalome resulting from molecular diagnostics threatens to undermine the promise of molecular medicine in at least three ways (Kohane et al. 2006):

1. Physicians will be overwhelmed by the complexity of pursuing unexpected genomic measurements.
2. Patients will be subjected to unnecessary follow-up tests, causing additional morbidity.
3. The cost of genomic medicine will increase substantially with little benefit to patients.

Given the current limitations of sensitivity and specificity of many genomic tests, application of these for screening of large populations to detect conditions with low prevalence will result in large numbers of false positives. Even if genomic tests were to achieve 100 % sensitivity and a false-positive rate of zero, the risk of the incidentalome still remains. Some pathology of disease discovered incidentally never reaches clinical significance and may not influence decision for management. For example, a large number of prostate carcinomas accurately diagnosed after the finding of an elevated prostate-specific antigen level in all likelihood would not contribute to an individual's death and may not be treated.

The role of a genome-wide panel (i.e., a panel of 500, 000 genetic polymorphisms all ordered and measured together), however cost-effective to measure, needs to be compared with a series of more focused genomic-based panels with clear indications for use and proper protocols for workup of unexpected findings. The physicians need to be educated to ensure that there is appropriate clinical justification to perform and interpret these tests in a manner that ushers in the era of personalized medicine and does not allow the incidentalome to block its arrival.

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# Chapter 21
# Ethical Aspects of Personalized Medicine

## Introduction to Ethical Issues

Most of the ethical aspects of personalized medicine are based on pharmacogenetics, genetic screening and impact on healthcare. Understanding the social effects of genomics requires an analysis of the ways in which genetic information and a genetic approach to disease affect people individually, within their families and communities, and in their social and working lives. This information will lead to measures for the prevention of stigmatization and discrimination of different populations on ethnic grounds.

## Ethical Issues of Pharmacogenetics

Some of the ethical questions raised by pharmacogenetics include the following:

- The issue of ensuring equality in medical care, when genetics can predict which patients are less likely to benefit from the available pharmacotherapy.
- Another dilemma would be the right to deny an available treatment to specific patient populations according to information derived from pharmacogenetic studies.

The Nuffield Council on Bioethics in a report published in 2003 has reviewed this topic (www.nuffieldbioethics.org/pharmacogenetics). The report addressed a number of difficult questions, ranging from consent and confidentiality of the genetic information yielded from the tests to whether the tests should be available over the counter or through the Internet. It raised concerns that pharmacogenetics may cause inequality in health care and that patients may be subdivided according to racial or ethnic categories. The working party concluded that because there is considerable genetic variation within ethnic groups it is highly unlikely that being in a particular group could be used to determine whether or not a patient takes a pharmacogenetic test. However, the report recommended that pharmacogenetic tests be validated in

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the populations in which they are to be used and the delivery of pharmacogenetic testing should be made as straightforward as possible. Needs of healthcare professionals as well as patients for access to reliable information about tests and medicines from independent sources were emphasized. Family physicians will need guidance in answering new types of question, such as whether patients should be entitled to a prescription for a drug even if they do not wish to take an associated test.

In case the safe and effective use of a medicine can only be determined by pharmacogenetics, bypassing of the test would subject the patient to risk and should not be permitted. There is too much fuss being made about the ethical aspects of genetic information. It is no different from other laboratory parameters of a patient with interindividual differences.

## Ethical Aspects of Genetic Information

### *Ethical Issues of Whole Genome Analysis*

The ability to sequence an individual's entire genome will enable production of an unprecedented amount of detailed genetic information, helping researchers to explore the relationship of genes and environment in the development of a wide variety of human diseases. Researchers would be seeking to produce a record of all the genetic information of subjects. As a result, all known genetic predispositions will be available and, depending on the data sharing policy, accessible to a wide range of researchers and, possibly, the public at large. This will raise ethical issues about access to and use of genetic information. In order to live up to its potential, whole-genome research in the future should be built upon some ethical foundation that will give people the confidence and trust they will need in order to become volunteers. A group of experts has published a statement of consensus that is intended to serve as practical guidance for scientists involved in whole-genome association research and for ethics boards (Caulfield et al. 2008). Although there is an immediate need for ethics guidance, the research communities also should continue to explore the ethical, legal, and social implications of this rapidly evolving field.

The ethical framework needed to encourage individuals to join whole-genome association studies, should support good policies for consensual use of personal information, allow individuals freedom to withdraw from research, provide guidance for what type of information should be offered to participants, and should help guide and control the public release and storage of whole-genome association data. The statement proposes eight recommendations aimed at creating more secure and consensual practices for research institutions involved in whole-genome association studies. Among their suggestions, the authors propose that before beginning participation in a whole-genome association study, participants should be asked to provide consent for future use that includes as much detail as possible, including information about the sampling and sequencing process, associated commercialization activities, possible risks, and the nature of likely future research initiatives. This process should

cover information about data security and about the governance structure and the mechanism for considering research protocols in the future. The right to withdraw consent at any time, for any reason, and without repercussions is a central component of existing research ethics statements. That right, which must include the destruction of tissue samples and written information, must, so far as possible, be respected and be part of the whole-genome research ethics process. In addition, the fact that this right may be severely limited once data are disseminated must be clearly communicated as part of the initial informed consent process.

Scientists also must look into the connection between how data and samples are collected, stored, and disseminated and the participant's ability to withdraw from subsequent use. This issue will need to be considered on a case-by-case basis per project. In addition, the process of disclosing results to participants should provide them with sufficient interpretive information. These results should be scientifically valid, confirmed, and should have significant implications for the subject's health and well-being. The studies also should be structured with plans to return other forms of significant non-health-related data as well. Data-release policies must balance the benefits and requirements of access and privacy interests, and the rationales for these policies must be explained, justified, and considered acceptable by an ethics review entity. For potential participants in whole-genome association studies, the implications of this data release must be disclosed, and the finality of the release process and its potential implications on privacy must be explained to the participant.

## *Ethical Aspects of Direct-to-Consumer Genetic Services*

Advertising for direct-to-consumer (DTC) genetic screening tests that lack independent professional oversight raises troubling questions about appropriate use and interpretation of these tests by consumers and carries implications for the standards of patient care (Geransar and Einsiedel 2008). Concern has been expressed that these premature attempts at popularizing genetic testing neglect key aspects of the established multifaceted evaluation of genetic tests for clinical applications and could confound treatment or complicate doctor-patient relations (Hunter et al. 2008).

A statement released by the American College of Medicine Genetics Board of Directors in 2003 states: "Genetic tests of individuals or families for the presence of or susceptibility to disease are medical tests. At the present time, genetic testing should be provided to the public only through the services of an appropriately qualified health care professional. The health care professional should be responsible for both ordering and interpreting the genetic tests, as well as for pretest and posttest counseling of individuals and families regarding the medical significance of test results and the need, if any, for follow-up. Due to the complexities of genetic testing and counseling, the self-ordering of genetic tests by patients over the telephone or the Internet, and their use of genetic "home testing" kits, is potentially harmful. Potential harms include inappropriate test utilization, misinterpretation of test results, lack of necessary follow-up, and other adverse consequences."

A commentary in the Journal of American Medical Association offers several caveats and recommendations to help doctors and counselors as they consider offering these research-based tests in clinical practice (Offit 2008):

- There is concern about the scientific accuracy of some of these tests, because they have not yet been validated in prospective clinical studies. In addition, the laboratory accuracy of these tests may vary.
- Direct to consumer aspect of the marketing of these tests excludes guidance from healthcare professionals. This limits the sources of information available to consumers about these tests and their accuracy from those marketing the tests. This critical lack of information raises concerns that patients/individuals may not have the resources to make unbiased decisions regarding whether to proceed with genetic testing.
- Once these self-ordered test results are relayed, individuals receiving the results may not receive counseling regarding appropriate medical interventions for prevention and early detection of genetic disorders.

A follow-up of selected sample subjects who went DTC genomewide testing, there were no measurable short-term changes in psychological health, diet or exercise behavior, or use of screening tests (Bloss et al. 2011). A study by the Mayo clinic showed that predictive genomic risk information from DTC testing modestly influences risk perception and worry; the extent and direction of this influence may depend on the condition being tested and its baseline prominence in preventive health care, which may attenuate with time (James et al. 2011). The concern of the medical profession about the potential harm of DTC is reflected in the following comments (Annes et al. 2010):

- Most genetic screening currently cannot meet the expectations of established principles in order to avoid undue harm and expense and may place a substantial burden on the health care system without providing demonstrable benefit.
- US Government Accountability Office's investigative report in 2010 cited erroneous medical management advice from DTC genetic-testing companies, and a lack of standardization of results – clinically valid tests for the same condition should yield concordant results.
- Potential harms of DTC genetic testing include the loss of protections for patients offered by established health care delivery systems such as doctor–patient confidentiality, invalid analytic or clinical results from medical devices, and population screening without consensus on interpretation and follow-up.

## *Privacy Issues in Personalized Medicine*

Genetic tests challenge privacy depending on how comprehensive the test is and how the access to samples or digital information is controlled. Point-of-care (POC) tests are likely to be limited in scope, fit seamlessly into medical records and do not raise new ethical and privacy challenges. Large-scale clinical trials, on the other hand,

result in large databases of genomic information. The magnitude of the genomic scans, implications of the inclusion of genetic information about relatives, security of storage and ease of dissemination of data present greater challenges to privacy compared to traditional, self-limited and often transient medical information.

## *Genetic Information Nondiscrimination Act in the US*

In 2008, the US Congress passed the legislation, known as the Genetic Information Nondiscrimination Act (GINA), which prohibits the following (Hudson et al. 2008): (1) group and individual health insurers from using a person's genetic information in determining eligibility or premiums; (2) an insurer from requesting or requiring that a person undergo a genetic test; and (3) employers from using a person's genetic information in making employment decisions such as hiring, firing, job assignments, or any other terms of employment. GINA does not prevent health care providers from recommending genetic tests to their patients or mandate coverage for any particular test or treatment.

As a result of GINA, more people are expected to take advantage of genetic testing and to participate in genetic research. However, the health insurance measure would not go into effect until a year after, and the employment measure would take effect only after 18 months. Even then, there may be reason to be cautious. The bill may be hard to enforce and it does not address discrimination by long-term care insurers or life insurers. The use of genetic information that the bill is likely to encourage may raise still more questions about how it should be used. These protections offered by GINA do not, however, extend to the disease manifestations of genetic risks. Although genomic information showing a predisposition to cancer would be protected under GINA, other clinical signs or symptoms indicative of cancer are not protected. Provisions of the Affordable Care Act set to go into effect in 2014 go a step further and will preclude consideration of all preexisting conditions, whether genomic or not, in establishing insurance premiums. Current federal laws, however, do not restrict the use of genomic information in life insurance, long-term care insurance, or disability insurance.

## *Genotype-Specific Clinical Trials*

Genotype-specific clinical trials would likely include subjects likely to respond to a drug. The inclusion of subjects known to be unlikely to respond would pose ethical problems:

- Genetic variations of pharmacological significance among ethnic groups might be a barrier to participation in clinical trials for fear of stigmatization
- Genetic testing of populations as a part of development of personalized medicine raises ethical issues

- Genetic information about the patient, confided only to the physician in traditional medicine, will be accessible to other healthcare personnel in clinical trials of personalized medicine, e.g. pharmacists.

## Social Issues in Personalized Medicine

Introduction of personalized medicine in healthcare systems of Western cultures would need to fulfill requirements of basic social values. Pharmacogenomics with genotype-based optimization of therapeutic interventions would need to demonstrate the following:

- Individual's freedom of choice is not restricted by information generated by pharmacogenomics.
- Access to novel medical applications stemming from pharmacogenomics is granted to all social and ethnic segments of the society.
- The patient has full control over all his/her individual data.
- Novel therapeutic approaches are in no way hazardous to the patient.

It is now well documented that substantial disparities exist in the quality and quantity of medical care received by minority Americans, especially those of African, Asian and Hispanic heritage. In addition, the special needs and responses to pharmaceutical treatment of these groups have been undervalued or ignored. Genetic factors underlie varying responses to medicines observed among different ethnic and racial groups. Pharmacogenetic research in the past few decades has uncovered significant differences among racial and ethnic groups in the metabolism, clinical effectiveness, and side-effect profiles of many clinically important drugs. These differences must be taken into account in the design of cost management policies such as formulary implementation, therapeutic substitution and step-care protocols. These programs should be broad and flexible enough to enable rational choices and individualized treatment for all patients, regardless of race or ethnic origin.

## Race and Personalized Medicine

Pharmacogenetics is growing fast and has reopened the debate on the biological basis of race and ethnicity. It is hoped that and it will lead to a more refined understanding of ethnic and racial differences in drug response. In spite of the contentious nature of discussions about human races, it is often assumed that racial categorization has clinical relevance when it comes to the choice of drug therapy. Chinese patients require lower dosages of heparin and warfarin than those usually recommended for Caucasian patients. As mentioned in Chap. 7, there are race-specific therapies for cardiovascular disease. Randomized trials have been interpreted to show that a combination of

vasodilators is more effective in treating heart failure in black persons than in white persons and that ACE inhibitors have little efficacy in blacks.

In order to address the health concerns of blacks in the US, Howard University (Washington, DC), a historically black institution, started to create the nation's largest repository of DNA from African-Americans in 2003. The samples were used to find genes involved in diseases with particularly high rates among blacks, e.g. hypertension and diabetes. Over a 5-year period, blood samples or cheek swabs were gathered from 25,000 persons, mainly patients at hospitals associated with the Howard College of Medicine. The genetic information would help to find the cause of a disease, predict susceptibility to an illness and help to choose a drug that would work best for a particular patient.

Race is frequently used by clinicians to make inferences about an individual's ancestry and to predict whether an individual carries specific genetic risk factors that influence health. The extent to which race is useful for making such predictions depends on how well race corresponds with genetic inferences of ancestry. Recent studies of human genetic variation show that while genetic ancestry is highly correlated with geographic ancestry, its correlation with race is modest. Because of substantial variation within human populations, it is certain that labels such as race will often be an inaccurate proxy when making decisions about disease predisposition and drug response. Because data on the correspondence of race, ancestry, and health-related traits are limited, particularly in minority populations, geographic ancestry and explicit genetic information are alternatives to race that appear to be more accurate predictors of genetic risk factors that influence health and should be considered in providing more personalized health care.

However, the public health relevance of various studies remains controversial. Many researchers and policy makers argue against the use of racial or ethnic categories in medicine, saying that classifying people according to race and ethnicity reinforces existing social divisions in society or leads to discriminatory practices. Race has not been shown to provide a useful categorization of genetic information about the response to drugs, diagnosis, or causes of disease. The current concept of race is a social construct defined by geography and culture with no genetic basis. There are no genetic variants that are found in every member of one race and none of another. Risk factors associated with race are not exclusive and may be found in several different races. There are biological variations among people but they may not parallel the categories of races as practiced now.

There are racial and ethnic differences in the causes, expression, and prevalence of various diseases. The relative importance of bias, culture, socioeconomic status, access to care, and environmental and genetic influences on the development of disease is an empirical question that, in most cases, remains unanswered. Nevertheless ignoring racial and ethnic differences in medicine and biomedical research will not make them disappear. Rather than ignoring these differences, scientists should continue to use them as starting points for further research. Only by focusing attention on these issues can we hope to understand better the variations among racial and ethnic groups in the prevalence and severity of diseases and in responses to treatment.

ApoEε4 confers a risk of Alzheimer's disease in a population-specific manner. As compared with the risk among those who do not carry an ApoEε4, the risk conferred by homozygosity for this allele is increased by a factor of 33 among Japanese persons, a factor of 15 in white populations, and by a factor of 6 among black Americans. These increases indicate that there are modifying effects on ApoEε4–mediated susceptibility in these populations, that other gene variants that are more important than ApoE in conferring risk are enriched or depleted in these populations, or that both are true.

A study has compared the incidence of coronary heart disease (CHD) over a 15-year interval in the Atherosclerosis Risk in Communities study according to the presence or absence of sequence variants in the proprotein convertase subtilisin/kexin type 9 serine protease gene (PCSK9) that are associated with reduced plasma levels of LDL cholesterol (Cohen et al. 2006). In black subjects examined, 2.6 % had nonsense mutations in PCSK9 associated with a 28 % reduction in mean LDL cholesterol and an 88 % reduction in the risk of CHD. In white subjects examined, 3.2 % had a sequence variation in PCSK9 that was associated with a 15 % reduction in LDL cholesterol and a 47 % reduction in the risk of CHD. In this study, the race question proved decisive. The researchers found that these relatively rare alleles correlated with low LDL, and did so in both blacks and whites, allowing them to conclude that it was the gene change that was crucial. If the team had ignored race and simply compared those who had heart disease with those who did not, and asked which alleles were linked to the risk, they would probably have missed the clinical significance of the alleles. This is because they would have appeared so infrequently – in less than 0.3 % of the whole study population for version 142X – that their effects would have been swamped. That is even truer for less populous racial groups; indeed, the smaller the group, the less likely researchers are to find important but rare alleles unless they can break the population down. Ignoring race altogether would be to the detriment of medical knowledge about the very people who might benefit.

Inflammatory bowel disease (IBD) affects American Jews of European descent 2–3 times more frequently than other ethnic groups. However, IBD is being diagnosed with increasing frequency now in Hispanics and African-Americans. One of the explanations for these disparities is that most diseases are not single-locus genetic diseases and environmental factors also play a role in the causation of disease.

It is because of the potential usefulness of gene variants in predicting risk and targeting therapies that the quest for genes that underlie complex traits continues. The goal of personalized medicine is the prediction of risk and the treatment of disease on the basis of a person's genetic profile, which would render biologic consideration of race obsolete. But it seems unwise to abandon the practice of recording race when we have barely begun to understand the architecture of the human genome and its implications for new strategies for the identification of gene variants that protect against, or confer susceptibility to, common diseases and modify the effects of drugs.

Although past studies have shown that genomic diversity and allele frequency patterns vary by population, those based solely on self-reported ancestry often do not reflect genetic ancestry and exclude individuals who are of mixed ancestry.

Genomic information is now increasingly replacing self-reported race in medical- and population-related research. With the availability of markers in population genetics that are informative of ancestry and reveal genetic clues, the concept of race is no longer useful in the context of this research.

## Gene Patents and Personalized Medicine

Gene patents for therapeutics have often been subject of litigation but there is surprisingly little publicity. In contrast, genetic diagnostics have been highly controversial but rarely litigated until now. Problems do occur when patents are exclusively licensed to a single provider and no alternative is available. Courts have been changing the thresholds for what can be patented, and how strongly patents can be enforced. Technologies for sequencing, genotyping and gene expression profiling promise to guide clinical decisions in managing common chronic diseases and infectious diseases, and will become an integral part of personalized medicine. Developing such genomic tests may require exploring a complex landscape of intellectual property and cutting through thickets of patented DNA sequences. A study found that patent claims, if strictly enforced, might block the use of multi-gene tests or full-genome sequence data (Chandrasekharan and Cook-Deegan 2009). With availability of new technologies that reduce the costs of complete genomic sequencing to prices that are comparable to current genetic tests, policy makers and courts are unlikely to allow intellectual property to obstruct such technological advance, but prudent policy will depend on careful analysis and foresight.

In 1996, Myriad Genetics in the US began offering genetic diagnostic tests for mutations in the genes BRCA1 and BRCA2, which are linked to hereditary breast and ovarian cancer. Since that time, Myriad has been a forerunner in the field of personalized medicine through the use of effective commercialization strategies which have been emulated by other commercial biotechnology companies. Myriad's strategies include patent acquisition and active enforcement, direct-to-consumer advertising, diversification, and trade secrets. These business models have raised substantial ethical controversy and criticism, often related to the company's focus on market dominance and the potential conflict between private sector profitability and the promotion of public health. However, these strategies have enabled Myriad to survive the economic challenges that have affected the biotechnology sector and to become financially successful in the field of personalized medicine. A critical assessment of the legal, economic and ethical aspects of Myriad's practices over this period allows the identification of the company's more effective business models (So and Joly 2013). The authors also discuss the consequences of implementing economically viable models without first carrying out broader reflection on the socio-cultural, ethical and political contexts in which they would apply.

On 13 June 2013, in "Association for Molecular Pathology vs Myriad Genetics Inc", the US Supreme Court unanimously ruled that naturally occurring genes cannot be patented, but synthetic transcripts of genes can be. The case involved patent claims

covering BRCA1 and BRCA2; mutations in these genes are linked to an increased risk for breast and ovarian cancer. Both sides quickly claimed victory. According to the plaintiffs, the Court's decision would help society "feel more of the impact of the genomics revolution", whereas the Biotechnology Industry Organization claimed that the decision left intact patents on the synthetic transcripts, "the commercially most important form of DNA used in biotechnology" (Sherkow and Greely 2013).

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# Chapter 22
# Regulatory Aspects of Personalized Medicine

## Introduction

The regulatory agencies have not laid down any specific guidelines for the personalized medicines. Most of the discussion relevant to this topic is covered under the overlapping components of personalized medicine: pharmacogenetics, pharmacogenomics, molecular diagnostics, and companion diagnostics. Accuracy, sensitivity and reproducibility are required for any diagnostic procedure that is to be used for predictive drug testing. It is desirable that companies developing genetic test methods should be certified for their capabilities for detecting a genotype variant or a SNP from any given patient sample. Only after confirmation of the identity of the polymorphism, should the company be allowed to proceed to the next step of analysis, which involves proteomics or analysis of protein expression of the genotype variant. Pharmacogenomic testing may be used in clinical trials of a drug, in reevaluation of a failed drug candidate or for evaluation of patient responsiveness to a marketed drug. The quality of such testing is not yet adequately covered by the regulatory agencies. Regulatory agencies will need to apply new approaches towards the review and approval of molecular diagnostic tests that use new technologies as well as drugs that work in concert with companion diagnostics, often using complex multianalyte test formats. The information revealed by pharmacogenomic testing during drug development and that based on study of marketed drugs might reveal potential hazards that need to be included in the labeling, which currently includes only known hazards. Labeling should disclose not only risk information on the extrapolation of in vitro pharmacogenomic testing and in vivo drug responsiveness but also the recommended dose based on stratified patient groups according to genotype/phenotype profiles.

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## FDA and Personalized Medicine

Consistent with its core mission, the FDA's is working in collaboration with researchers, drug manufacturers, medical devices and biologics, health care professionals and others to better understand and adapt to the promise of personalized medicine (http://www.fda.gov/scienceresearch/specialtopics/personalizedmedicine/default.htm).

The FDA provides guidance on a broad range of topics, such as incorporating genetic and other biomarker information in drug development programs, designing clinical trials to incorporate biomarker data, coordinating cross-labeling activities, evaluating pharmacogenomics data, and demonstrating companion diagnostic test performance. FDA's ongoing efforts to make personalized medicine possible touch on various facets of product development and use including:

- Early stage development
- Regulatory pathways and policies
- Product use

### *Regulatory Aspects of Pharmacogenetics*

The attitude of various regulatory agencies to pharmacogenetics has so far been not been well defined. New regulatory challenges will surface with the development of drugs targeted at special populations. There are no regulatory requirements for pharmacogenetic data. Current guidelines of the European Medicines Evaluation Agency do not specifically mention pharmacogenetics but they recommend the value of a "population approach" to clinical trials to screen for drug interactions. The FDA is starting to formulate a policy on pharmacogenomic studies.

FDA currently views genetic variations as one of the many factors that contribute to drug response and a 1999 document by the FDA on drug metabolism/interactions in vitro refers to use of pharmacogenetic data in determining drug dosage: "In vivo drug metabolism/drug interaction studies" (www.fda.gov/cber/guidelines.htm). One example quoted in this draft is that if in vitro studies indicate that CYP2D6 or 3A4 enzyme systems do not metabolize an investigational drug, then clinical studies to establish this effect are not necessary. The FDA occasionally has used early pharmacogenomics information on a drug's label. For example, the drug Straterra, for attention deficit and hyperactivity disorder, contains information that people with a variation of the 2D6 drug-metabolizing enzyme process the drug more slowly and thus are more prone to side effects. Some children with leukemia have an enzyme deficiency that makes the standard therapeutic dose of mercaptopurine far too high for their bodies. The FDA's scientific advisers have recommended adding that information to the drug's label, too. There is a need for good studies on this topic. As personalized medicine gets established, it is expected that the regulatory agencies will work on guidelines for this system, e.g., approval of drugs packaged with diagnostic tests.

## *FDA and Pharmacogenomics*

Pharmacogenomics is an area of development the FDA views very positively. The FDA has received numerous drug submissions that included pharmacogenomic data since issuing guidance on how to do so in 2005. The most recent is 2013 guidance document "Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling". This guidance is intended to assist the pharmaceutical industry and other investigators engaged in new drug development in evaluating how variations in the human genome, specifically DNA sequence variants, could affect a drug's pharmacokinetics, pharmacodynamics, efficacy, or safety. It provides recommendations on when and how genomic information should be considered to address questions arising during drug development and regulatory review, focusing on general principles of study design, data collection, and data analysis in early-phase trials. It also provides recommendations for labeling.

Several cancer drugs have been approved that include pharmacogenomic data for the guidance of physicians prescribing these drugs. The FDA also is exploring similar guidelines for pharmacoproteomic data.

### FDA Guidance for Pharmacogenomic Data Submissions

The updated guide to pharmacogenomic data submission was issued by the FDA in March 2005. Current information relevant to pharmacogenomics is available on FDA's website (http://www.fda.gov/cber/gdlns/pharmdtasub.htm). In a press in 2005, the FDA recognized that pharmacogenomics allows health care providers to identify sources of an individual's profile of drug response and predict the best possible treatment option for this individual. FDA's efforts in this direction will facilitate the development of personalized medicine. FDA's guidance "Pharmacogenomic Data Submissions," clarifies how pharmacogenomic data will be evaluated. The final guidance describes what data will be needed during the marketing application review process, the format for submissions, and the data that will be used during regulatory decision making. The guidance also explains a new mechanism for industry to voluntarily submit research data to further the scientific exchange of information as we move into more advanced areas of pharmacogenomic research. The voluntary data, which will be reviewed by an internal, agency-wide group and will not be used for regulatory decision making, will help FDA and industry gain valuable experience as this new field continues to evolve.

FDA believes this approach will save time and resources and eliminate possible delays in the application review process because parties will be able to familiarize themselves with novel pharmacogenomic approaches as they evolve.

FDA has already received several pharmacogenomic data submissions through both the regulatory and voluntary processes and will continue to work closely with industry and the healthcare community on this exciting emerging technology.

The FDA believes that pharmacogenomic testing can be smoothly integrated into drug development processes. Currently, scientific understanding of pharmacogenomics is most advanced in the drug metabolism area, and early results are expected in this field. However, FDA anticipates rapid evolution of additional uses. For example, it is hoped that pharmacogenomic testing will help identify cancers that have a high probability of responding to a particular medication or regimen. Pharmacogenomics may also be used to help track down the cause of certain rare, serious drug side effects.

The guidance provides specific criteria and recommendations on submission of pharmacogenomic data INDs and NDAs and Biological License Applications (BLAs). This includes information on what data is needed, and how FDA will or will not use such data in regulatory decisions. Because there is a need for scientific exchange, the agency is asking for voluntary submissions of research information. This data will help FDA gain experience as the field evolves. In these cases, FDA advises sponsors to clearly label voluntary submissions; and the agency advises that it will not use information from voluntary reports for regulatory decisions. If a sponsor subsequently develops additional data that meet the criteria for submission for regulatory purposes, the Agency advises sponsors that such data should be submitted as explained in the guidance.

### Joint Guidelines of the FDA and EU Regulators for Pharmacogenomics

In 2006, the FDA and European drug regulators agreed to a joint procedure that pharmaceutical companies can follow to voluntarily submit pharmacogenomic data to both agencies. The document, which can be assessed at the web site of EMEA (http://www.emea.eu.int/), could benefit pharmaceutical companies interested in simultaneously selling products in Europe and the US that have pharmacogenomic components. Specifically, the European Medicines Agency and the FDA released a set of "guiding principles" describing how they will process drug developer's requests to jointly meet with both agencies about voluntary genomic data submission. The guiding principles have a list of definitions agreed to by the agencies, and a flowchart describing how voluntary submissions would be processed. The FDA's Interdisciplinary Pharmacogenomic Review Group and the EMEA's Pharmacogenetics Working Party will review the data submission packages.

### Pharmacogenomic/Pharmacogenetic Information in Drug Labels

Currently, there are >50 drugs with pharmacogenetic discoveries on their labeling, which can be accessed at: www.accessdata.fda.gov/scripts/cder/drugsatfda. The FDA wants to see genomic information front and center in a drug's label. The agency has released guidelines for the "Clinical Pharmacology Section of Labeling for New Prescription Drugs, Content and Format". The format for new drug labels includes a pharmacogenomics section, and will relocate pertinent genetic information to a box

at the top of the label. With a pharmacogenomics section in new labels, FDA is planning ahead to reserve a spot in the label that is specifically intended for pharmacogenomic information that comes out of drug development or that comes out of post-marketing studies. In the past, genomics information was part of a drug's pharmacokinetic and pharmacodynamic profile and appeared in the pharmacology section, lost within the lengthy and text-heavy product labels. FDA wants to improve on the location of clinically relevant genetic information in the label. Examples of genetic information labels for some drugs are shown in Table 22.1.

**FDA Guidelines for Pharmacogenomics-Based Dosing**

According to a draft report entitled "Realizing the Promise of Pharmacogenomics: Opportunities and Challenges", issued by the Department of Health and Human Services (HHS) in 2007, the FDA must issue guidelines to help physicians use pharmacogenomics tests for drug-dosing before the clinical community can adopt them fully. Despite approval of Roche's AmpliChip and including genetic information in the label for Pfizer's colorectal cancer drug Camptosar, the FDA has not clarified how physicians should use the tests. Apart from FDA's role as market gatekeeper for pharmacogenomics products, FDA requirements and actions or the lack thereof, influence the ways in which marketed pharmacogenomic diagnostic technologies are used in clinical practice. For example, FDA approval of a pharmacogenomic test does not necessarily result in dosing guidelines for accompanying therapy. Pharmacogenomic-based testing can identify patients who are likely to respond differently to particular drugs and indicate the need for customized dosing, but that testing does not necessarily translate into dosing instructions. As such, patients will have to be monitored and have their dosing adjusted empirically.

## *FDA and Validation of Biomarkers*

The FDA has recognized pharmacogenomics as an opportunity to identify new biomarkers that may expedite the drug development process. FDA guidance also makes a distinction between pharmacogenomic tests that may be considered either probable or known valid biomarkers, which may be appropriate for regulatory decision making, and other less well-developed tests that are either observational or exploratory biomarkers that, alone, are insufficient for making regulatory decisions.

A pharmacogenomic test result may be considered a valid biomarker if it is measured in an analytical test system with well-established performance characteristics and there is an established scientific framework or body of evidence that elucidates the physiologic, pharmacologic, toxicologic, or clinical significance of the test results. For example, the effects of genetic variation in the human enzymes CYP2D6 and thiopurine methyltransferase on drug metabolism are well recognized scientifically and are included in some approved drug labels. The results of genetic tests that

**Table 22.1** Drugs with genetic information in their labels

| Drug | Company | Indication | Genotype | Effect | Label |
|---|---|---|---|---|---|
| Abacavir (Ziagen) | GlaxoSmithKline | HIV-1 | HLA-B*5701 | Hypersensitivity | Screening for the HLA-B*5701 allele should be done prior to initiating therapy with abacavir |
| Azathioprine (Imuran) | Prometheus | Renal allograft transplantation | TPMT*2, TPMT*3A, TPMT*3C | Severe myelotoxicity | TPMT genotyping can help identify patients who are at an increased risk for developing Imuran toxicity |
| Carbamazepine (Tegretol) | Novartis | Epilepsy, trigeminal neuralgia | HLA-B*1502 | Stevens-Johnson syndrome (toxic epidermal necrolysis) | Patients in genetically at-risk populations should be screened for HLA-B*1502 prior to initiating treatment and those testing positive not be treated with Tegretol |
| Cetuximab (Erbitux) | Imclone | Metastatic colorectal cancer | KRAS mutations | Efficacy | Use of Erbitux is not considered effective for the treatment of patients colorectal cancer who have KRAS mutations in codon 12 or 13 |
| Clopidogrel (Plavix) | Bristol-Myers Squibb | Anti-platelet: prevents strokes and heart attacks | CYP2C19*2*3 | Efficacy | Alternative treatment should be considered for patients identified as CYP2C19 poor metabolizers |
| Irinotecan (Camptosar) | Pfizer | Metastatic colorectal cancer | UGT1A1*28 | Diarrhea, neutropenia | A reduction in the starting dose by at least one level of Camptosar should be considered for patients known to be homozygous for UGT1A1*28 allele |
| Panitumumab (Vectibix) | Amgen | Metastatic colorectal cancer | KRAS mutations | Efficacy | Vectibix is ineffective in patients whose tumors have KRAS mutations |

distinguish allelic variants of these enzymes are considered to be well established and, therefore, valid biomarkers.

A probable valid biomarker is one that is measured in an analytical test system with well-established performance characteristics and for which there is a scientific framework or body of evidence that appears to elucidate the physiologic, toxicological, pharmacologic, or clinical significance of the test results. A probable valid biomarker may not have reached the status of a known valid marker because, for example, of any one of the following reasons:

- The data elucidating its significance may have been generated within a single company and may not be available for public scientific scrutiny.
- The data elucidating its significance, although highly suggestive, may not be conclusive.
- Independent verification of the results may not have occurred.

The distinction between what tests are appropriate for regulatory decision making and those that are not will change over time as the science evolves. Throughout the development of these tests, as appropriate, FDA will continue to seek public comment as we evaluate whether a biomarker is a valid biomarker (e.g., via discussions at Advisory Committee meetings).

Algorithms described in the FDA Pharmacogenomics Guide for investigational and marketing application holders describe when to submit to FDA data on known valid biomarkers. Data on probable valid biomarkers need not be submitted to the IND unless they are used by a sponsor to make decisions regarding specific animal safety studies or clinical trials (e.g., using biomarker data as inclusion or exclusion criteria, assessment of treatment-related prognosis, or stratifying patients by dose) or are a probable valid biomarker in human safety studies. However, FDA recommends that sponsors or applicants submit reports on all probable valid biomarkers to new (i.e., unapproved) NDAs or BLAs according to the algorithm. Many pharmacogenomic testing programs implemented by pharmaceutical sponsors or by scientific organizations are intended to develop the knowledge base necessary to establish the validity of new genomic biomarkers. During such a period of scientific exploration, test results are not useful in making regulatory judgments pertaining to the safety or effectiveness of a drug and are not considered known or probable valid biomarkers.

## *FDA and Predictive Medicine*

The FDA released a white paper in 2004 entitled “Innovation or Stagnation? Challenge and Opportunity on the Critical Path to New Medical Products” (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html). This white paper was a serious attempt by the FDA to bring attention and focus to the need for targeted scientific efforts to modernize the tools, techniques and methods used to evaluate the safety, efficacy and quality of drug products. It describes the urgent need for cooperation between the FDA, the NIH and the private sector to modernize the

development process for medical products – the Critical Path – to make product development more predictable and less costly. The critical path determines the potential bottlenecks in bringing a product to market. The focus of the Critical Path Initiative is to identify ways to update the product development infrastructure for drugs, biologics and devices, and the evaluative tools currently used to assess the safety and efficacy of new medical products. Examples of evaluative tools include the use and verification of pathophysiological and/or descriptive biomarkers for patient selection for clinical trials and/or use as surrogate endpoints. In addition, an important example of a scientific opportunity for improving the critical path is the use of pharmacogenomics and pharmacogenetics or, more specifically, the identification of DNA-based biomarkers or RNA-expression profiles that can provide insights into the stage of a disease, disease progression, drug response and drug-dosing requirements, and thereby lead to the development of tests to predict clinical outcomes more reliably.

### *FDA Regulation of Multivariate Index Assays*

In 2006, the FDA took a step toward regulating a new category of complex genetic diagnostic tests that are expected to play a growing role in tailoring medical treatments to specific patients. The FDA is calling these tests "multivariate index assays (MIAs)." According to the FDA, such tests require approval before they can be marketed to ensure that the tests are valid. The new policy, published as draft guidelines, is open for public comment and would also be a step toward expanding the FDA's oversight to clinical laboratories (http://www.fda.gov/cdrh/oivd/guidance/1610.html). The FDA published a notice of availability of a revised draft guidance, on "In Vitro Diagnostic Multivariate Index Assays" in the Federal Register of 26 July 2007 (72 FR 41081) and comments were invited. At this time Genentech filed a Citizen Petition with the FDA urging the agency to take on greater oversight of diagnostic tests that are intended to guide therapeutic decisions and to regulate all laboratory-developed tests. According to Genentech, pharmacogenomic information is contained on the label of ~10 % of all FDA-approved drugs. Included among those are Genentech's trastuzumab (Herceptin), which requires that patients be tested for particular genetic characteristics and the results be considered before the drug is administered.

Currently, tests developed and performed by a single laboratory, known as homebrew tests, have been generally considered laboratory services and outside FDA purview. Now the FDA will regulate at least one category of such tests: those that measure multiple genes, proteins or other pieces of clinical information taken from a patient and then use an algorithm or software program to analyze the data.

The best known of these tests is Oncotype DX (Genomic Health). It analyzes the activities of 21 genes in a sample of breast tumor and then computes a score that is said to be predictive of whether a patient's cancer will recur and whether she would benefit from chemotherapy. While there are only a few such complex tests on the market now, their number is expected to grow. For personalized medicine, a

combination of genes or proteins is a better indicator of disease or disease risk than a single gene or protein. FDA considers regulation of such tests because the algorithms used are usually proprietary, making it difficult for physicians to interpret the test results. Therefore, the agency needs to look at the data on which these tests are developed. The FDA would decide case by case what to do about the tests already on the market. Some might have to come off the market until the developer can provide enough data for approval. The FDA approach will meet the need for an oversight of genetic tests, which have proliferated and are becoming increasingly complex. Government agencies have been criticized for not doing more to clamp down on questionable genetic tests that are being sold directly to consumers.

Three components are needed to ensure the safety and quality of genetic tests: (1) the laboratories that conduct the tests must have quality control and personnel standards in place to prevent mistakes; (2) the tests themselves must be valid and reliable – i.e., detect genes that are actually related to disease or disease risk accurately over time; and (3) health care providers must understand when to order the tests, how to in interpret them, and what to do with the results. Once these mechanisms are in place, uses and outcomes also must be evaluated over time in order to pinpoint any problems that may require attention, particularly as new tests enter wider use.

However, the requirement could also discourage the development of diagnostics by raising the costs of introducing them. Requiring clinical trials and FDA approval would discourage development of tests, which do not usually command the same profits as drugs. The requirement could discourage gradual improvements of tests because each change in a test might require a new regulatory submission. The draft policy has raised speculation that the FDA will eventually move to regulate additional laboratory tests beyond the complex ones.

In 2007, the FDA classified gene expression-based breast cancer prognostic tests as Class II devices and released a "special controls" guidance for companies developing such tests. The document is designed was a prototype guidance to provide a general framework for how the FDA's Office of In Vitro Diagnostics approaches IVD MIAs. The FDA cleared the first such IVDMIA device – Agendia's MammaPrint test – in 2007. In a Federal Register notice in 2007, FDA explained that it had originally classified MammaPrint as a Class III device, which would have required full premarket approval, but Agendia filed a petition requesting that the device be reclassified into Class II, which only requires 510(k) premarket notification. The FDA determined that MammaPrint, as well as future genomic breast cancer prognostics tests, can be classified as class II devices with the establishment of special controls, which are outlined in the guidance document as follows: "Any firm submitting a 510(k) premarket notification for a gene expression profiling test system for breast cancer prognosis will need to address the issues covered in this special controls guidance," the agency said in the document. The recommendations in the guidance document apply to RNA expression assays used for cancer prognosis, including RT-PCR and gene expression microarrays, in which an algorithm is applied to such measurements to yield a result that can be used by physicians as a prognostic marker, in combination with clinicopathological factors, to assess the risk of cancer recurrence. The process for reviewing such tests is "contingent on the intended use of the device" therefore, design of studies and data sets required will be influenced

by a particular use. In this instance, a test for the prognosis of breast cancer would require different data than a test used to diagnose the disease. A number of tumor markers have already cleared as Class II devices.

## Evaluation of Companion Diagnostics/Therapeutic

Until recently there was no development pathway for FDA approval of the necessary companion diagnostic tests and their associated targeted therapies. In 2007, the Critical Path Institute (Tempe, AZ) used a $2.1 million Arizona state grant to work with the FDA and the NCI to standardize how companion diagnostics and therapies for cancer are evaluated. The goal of this collaboration was to establish the performance standards that would serve as the model for future FDA co-submissions of companion diagnostic tests and cancer drugs. The ultimate goal of the project is to guide the choice of targeted therapy so that patients receive the most effective treatments.

The FDA issued guidance for "In Vitro Companion Diagnostic Devices" on 6 August 2014 after the release of the draft in 2011.. The development of therapeutic products that depend on the use of a diagnostic test to meet their labeled safety and effectiveness claims has become more common. These technologies – including IVD companion diagnostic devices – are making it increasingly possible to individualize, or personalize, medical therapy by identifying patients who are most likely to respond, or who are at lower or higher risk for a particular side effect. This guidance defines IVD companion diagnostic devices, provides information for industry and FDA on possible premarket regulatory pathways and FDA's regulatory enforcement policy, and describes certain statutory and regulatory approval requirements relevant to therapeutic labeling.

The FDA recognizes that contemporaneous development of a therapeutic product and corresponding diagnostic device, although ideal, is not always possible. Along these lines, an IVD companion diagnostic device may be (1) a new device, (2) a new version of existing device, or (3) an existing device approved/cleared for another purpose. For a new device, the FDA recommends that a therapeutic product and its corresponding companion diagnostic should be developed and approved contemporaneously. In most cases, assuming device/test results are essential for product safety and efficacy, the FDA will not approve the product or use of the product with the device if the FDA has not also approved/cleared the device itself. However, the FDA has discretion to approve a therapeutic product for use with a companion device, even if the FDA has not yet approved/cleared the device. In this situation, the FDA expects it will approve/clear the device subsequently, and that sponsors will revise relevant therapeutic product labeling accordingly. The FDA will also consider whether additional protections are necessary.

In this context, the FDA refers to two scenarios where it may approve a therapeutic product for use with an unapproved/uncleared companion device: (a) a new product will treat a serious or life-threatening condition for which no satisfactory alternative treatment exists, and benefits from using the unapproved/uncleared companion

device outweigh risks; and (b) an already approved product exists, but labeling for that product must be revised to address serious safety concerns, and use of an unapproved/uncleared companion device may address such concerns.

If safe and effective use of a therapeutic product depends on use of a companion diagnostic device, the device should be available once the FDA approves the product. Thus, the FDA will apply a "risk-based approach" to determine regulatory pathways for IVD companion diagnostic devices, where the level of risk to patients and available controls to mitigate risks dictate whether the device will require PMA (premarket approval) or 510(k) clearance. Except in certain situations, such as (a) and (b) discussed above, the FDA intends to approve/clear a therapeutic product and its corresponding companion diagnostic device at the same time. Therefore, the FDA encourages sponsors to time clinical studies and regulatory submissions to facilitate concurrent approval.

In a situation where a relevant device is already legally marketed, but the device manufacture intends to market the device for a new use as a IVD companion diagnostic device for a new therapeutic product, the FDA considers this to be "a major change in the intended use of the device, raising new questions of safety and effectiveness." Thus, the FDA must approve/clear the new use of the device with the new product.

Regarding labeling of a relevant therapeutic product, the Draft Guidance notes that existing regulations (21 CFR 201.56 and 21 CFR 201.57) indicate that product labeling must include information relating to relevant laboratory tests. For example, if a therapeutic product is only safe and effective in a patient subpopulation identified by a diagnostic test, the Indications and Usage section of the labeling must define the patient subpopulation. Likewise, if a diagnostic test is essential for monitoring beneficial or adverse effects, the Warnings and Precaution section must identify the type of test. In addition, labeling must include information about the type of device (i.e., intended use of the device), rather than a specific manufacturer's device. Also, if the FDA approves/clears a companion diagnostic device after it approves a relevant therapeutic product, sponsors must update product labeling accordingly. Regarding labeling of IVD companion diagnostic device, the Draft Guidance states such labeling should specify the intended use of the device, as well as relevant therapeutic products. When appropriate, the labeling can name a class of therapeutic products, rather than specific products within the class. Device labeling should be expanded (i.e., approved/cleared) to reflect use in a new disease or setting, or with a different/new therapeutic product.

The FDA will consider any diagnostic device used to make treatment decisions in a clinical trial to be an "investigational device," unless the device is used in a matter already approved/cleared. A sponsor must comply with investigational device exemption (IDE) regulations that address significant risk devices, if one uses a diagnostic device to make critical treatment decisions, such as patent selection, treatment assignment, or treatment arm.

A diagnostic device and therapeutic product may be studied in the same investigational study, as long as the study otherwise meets IDE and IND regulations. Sponsors should include information about a planned use of an IVD companion diagnostic device and its use in clinical trials in an investigational submission.

Further, it will be helpful if the device sponsor and the therapeutic product sponsor submit information about the proposed IVD companion diagnostic device in a pre-IND submission. Finally, the FDA strongly encourages sponsors to request meetings with both relevant device and therapeutic product review divisions as early in development as possible.

## CLSI Guidelines for RNA Controls in Gene Expression Assays

Microarray and realtime quantitative PCR (qPCR) technologies are emerging as vital components of genomic, evidence-based medicine. Standard controls are required to ensure reliability and quality from these assay platforms before microarray and realtime-qPCR results are accepted for clinical applications. The ability to report reliable gene expression results of known quality is key to the successful employment of microarrays and realtime-qPCR as tools in toxicogenomics, pharmacogenetics, pharmacogenomics, and as diagnostic devices in clinical medicine.

In response to this need, Clinical and Laboratory Standards Institute (CLSI) has published "Use of External RNA Controls in Gene Expression Assays; Approved Guideline (MM16-A)", which provides a set of agreed-upon protocols supporting the use of external RNA controls in microarray- and realtime-qPCR-based gene expression experiments. This guideline addresses important issues associated with the use of external RNA controls as a tool for verification of technical performance. In addition, it supports the evaluation of qualitative results for a specific clinical analyte, including:

- Preparation of control transcripts
- Design of primers and amplicons
- Quality control
- Use in final experimental or clinical test application
- Analysis and interpretation of data obtained

This document is intended to help ensure comparable within-platform assay performance to enable comparisons of gene expression results. The protocols will enable research and clinical laboratories, regulatory agencies, accrediting agencies, reference laboratories, as well as test, microarray, and reagent manufacturers to assess the performance of these expression assays. Further details can be seen at CLSI website (http://www.clsi.org).

## Regulation of Direct-to-Consumer Genetic Testing

Various states are beginning to tackle the problem of uncontrolled personal genetic services. In 2008, New York State, warned 23 companies that they must have permits to offer their services to New Yorkers. New York's warning letter was a blow not only to new companies such as Navigenics (now acquired by Life Technologies) and 23andMe that entered into the field of consumer genomics in 2007, but also to

technology suppliers Affymetrix and Illumina, which make the tools the testing companies use. In 2008, Department of Health of the State of California, in an effort to prevent consumer genetic testing companies from offering their services to the state's residents, sent letters to 13 firms saying they are violating state law. One offense that genetic testing companies could commit would be to sell their products to California citizens over the Internet without the request or counsel of a physician. Another problem is that the companies' tests have not been validated for accuracy or for clinical utility, which is required under California law.

## *Need for Regulatory Oversight of DTC*

Within the genetic testing system, there are still questions about science, access, reimbursement, coverage, and oversight. The Genetic Alliance, a nonprofit health advocacy organization committed to transforming health through genetics, has suggested that informed decisions must be made on the basis of analytic and clinical validity, clinical utility, and individual usefulness, as well as an understanding of oversight, regulation, and reimbursement (Zonno and Terry 2009). Accurate, reliable, and validated information must be available to individuals and providers as they make decisions about testing and the information gained through the testing process. To maintain credibility and independence, a mandatory registry should be compiled and managed by a federal regulatory body, such as the FDA. Education regarding basic genetics and the testing process; professional society recommendations and guidelines, information for patients and providers on risk or diagnosis; and referral networks for specialists, researchers, and disease-specific organizations could all be built into or linked with the registry. Such a system would be transparent and coordinated with all stakeholders and agencies to balances safety, innovation, ethical and social issues.

Greater regulation is required to oversee the accuracy and quality of direct-to-consumer (DTC) genetic testing. Not doing so runs the risk of dangerously reassuring some and needlessly aggravating the already worried. Certain state health departments, e.g. that of New York, have indicated that genetic testing for disease risk must be requested by a licensed healthcare professional and must be performed in an approved clinical laboratory. In 2010, Navigenics (acquired by Life Technologies in 2012) received a license to offer its personal genomics services to residents of New York State.

There are three important issues that consumer genomic testing needs to address before it can become part of medical care:

- Analytic validity. A small error rate in sample can result in hundreds of misclassified variants for any individual patient.
- Clinical validity. Many complex diseases are caused by multiple gene variants, and interactions between variants and environmental factors, which are not known yet.
- Clinical utility. Few observational studies and almost no clinical trials demonstrate the risks and benefits associated with screening for individual gene variants.

Ensuring that the public has information adequate to making informed choices about genetic testing is a prerequisite to realizing the public health benefits that have been promised from genetic medicine. In order to get a better picture of the state of the new DTC genetic testing industry, how it works, and what buyers expect from these services, the National Human Genome Research Institute asked the Genetics and Public Policy Center (GPPC) at Johns Hopkins University to conduct studies under a grant awarded in 2008. The issues being studied relate particularly to the ways in which offering genotyping tests and services directly to customers by companies such as Decode Genetics, and 23andMe, differs from genetic testing offered by healthcare providers. GPPC is analyzing the current regulations that cover marketing, advertising, and selling of genetic testing directly to consumers, and trying to evaluate the validity of the claims sellers make in their advertising by comparing them to scientific literature. Another important question is how the utility of a DTC test can be measured and if the presence of a genetic mutation that is linked with levels of risk or predisposition toward an illness is usable. The researchers at GPPC are also looking at how state laws attempting to cover this very new field allow some incoherence and lack of uniformity. The center also will conduct some legal analysis that supports coordinated efforts to protect consumers. The study will not be completed until sometime late in 2010.

According to a study by an international team of researchers from the UK, US, Australia, Austria, and the Netherlands anticipatory governance is premature without a better understanding of how SNP-based whole-genome information is used by, and what it means to, a wide range of users (Prainsack et al. 2008). The authors believe that DTC whole-genome tests should not necessarily be evaluated under the same regulatory frameworks used for traditional genetics. Although they did not advocate an unregulated genomics market, the authors urged regulators to wait until information is available on the effects of such tests before introducing regulation. For instance, the team noted that personal genomics is pushing the individualization of responsibility for health one step further, without necessarily providing clear information about how genetics ties into health and individual choices. Effective responses to this situation require clarification of the novel issues created by the convergence of information about health, consumer and lifestyle choices, and genealogy; novel relationships between geneticists, patients, consumers and corporate executives; and the continued intensification of collaboration, on both the research and the patient/consumer sides. In spite of the criticism of DTC testing, there are some positive aspects. A study has concluded that individuals who present to health care providers with online DTC genetics information may be among the most motivated to take steps toward healthier lifestyles (McBride et al. 2009). These motives might be leveraged by health care providers to promote positive health outcomes.

In 2010, Walgreens postponed its plans to sell personal genetic test kits from Pathway Genomics at its nationwide drug stores after the FDA intervened and issued the following statement: “Pathway Genomics has moved outside of the currently sanctioned boundaries for lab-developed tests by marketing a product in a retail store that asks consumers to collect a sample. These kits have not been proven safe, effective or accurate and patients could be making medical decisions based on

data from a test that has not been validated by the FDA". In 2010, the FDA sent letters to five companies – 23andMe, Navigenics (acquired by Life Technologies in 2012), DeCODE Genetics, Illumina and Knome – notifying them that they must submit their products for review or discuss with officials why their products do not require FDA approval. In its letter to 23andMe, the FDA said it wants to prevent consumers from being "misled by incorrect test results or unsupported clinical interpretations." However, the FDA did not say that such genetic testing services should be taken off the market. The five companies had mixed responses to the FDA's warning. 23andMe disagreed with the FDA's decision, while Knome welcomed the FDA's review. The FDA held a public meeting in 2010 to initiate a dialogue with stakeholders concerning the regulation of laboratory-developed tests.

Challenges faced by the introduction of personalized medicine include gaps in the oversight of genetic testing (including regulation of companies providing test interpretation services), ensuring that realistic claims are made in promotional materials for genetic testing, determining the appropriate role of new genomic technologies in patient care, ensuring the privacy of patients' genomic data, and improving insurance coverage and reimbursement for genetic services (Evans et al. 2010). The Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS) advises the US secretary of health and human services and on these issues. A 2008 SACGHS report identified multiple regulatory gaps in the oversight of genetic testing (http://oba.od.nih.gov/oba), and federal agencies have begun to address these gaps. For example, investigations by the Federal Trade Commission (FTC) of claims made by two nutrigenetics companies led to the discontinuation of the manufacturing and marketing of the MyCellf Program, which included a test kit and consultation service. SACGHS is also preparing a report on the need for genetics education for POC practitioners, public health officials, and consumers and has begun to explore the implications of affordable whole-genome sequencing.

To ensure that rapidly evolving genomic technologies are responsibly utilized and that their promise is not oversold to the public, it will be important to advocate for rigorous evaluations of the clinical validity and utility of genomic tests, as well as for adequate regulation that simultaneously preserves innovation. Clinicians, researchers, academics, the commercial sector, and the government must work together for realization of the remarkable potential of personalized medicine.

The European Society of Human Genetics (ESHG) believes that regulations are needed to prevent DTC predictive genomic services without well-reviewed clinical validity and utility from making it into the market or into clinical practice. It has developed the following policy on advertising and provision of predictive genetic tests by such DTC companies (European Society of Human Genetics 2010):

1. Clinical utility of a genetic test shall be an essential criterion for deciding to offer this test to a person or a group of persons.
2. Laboratories providing genetic tests should comply with accepted quality standards, including those regarding laboratory personnel qualifications.
3. Information about the purpose and appropriateness of testing should be given before the test is done.

4. Genetic counseling appropriate to the type of test and disease should be offered; and for some tests psychosocial evaluation and follow-up should be available.
5. Privacy and confidentiality of sensitive genetic information should be secured and the data safely guarded.
6. Special measures should be taken to avoid inappropriate testing of minors and other legally incapacitated persons.
7. All claims regarding genetic tests should be transparent; advertisement should be unbiased and marketing of genetic tests should be fair.
8. In biomedical research, health care and marketing, respect should be given to relevant ethical principles, as well as international treaties and recommendations regarding genetic testing
9. Nationally approved guidelines considering all the above-mentioned aspects should be made and followed.

According to a survey, the majority of clinical geneticists in Europe are concerned that DTC genetic tests are not clinically useful, and believe that sales of certain types of DTC tests should be more closely regulated or banned (Borry and Howard 2013). In their opinion, 90 % DTC tests for predictive disease risks based on genes should require medical supervision. They are concerned that DTCs lack clinical validity and utility. Of the 131 geneticists surveyed, 69 % felt that DTC genetic tests for prenatal gender should be banned, 63 % wanted to ban DTC genome scans, and 53 % believe that preconception disease carrier tests also should be banned. However, only 27 % wanted to ban DTC ancestry testing. The authors of the study concluded that better regulation is needed at the level of market introduction of DTC genetic tests and a procedure should be developed similar to that for pharmaceuticals.

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# Chapter 23
# Economics of Personalized Medicine

## Introduction

Success of personalized medicine cannot be measured in dollars alone. The improvement in healthcare and quality of life with reduction of disease burden will have an impact on all aspects of human life with economic benefits. A discussion of financial aspects, of personalized medicine, however, is important for two reasons: (1) pharmaceutical companies would like to know if it would be profitable; and (2) healthcare providers would like to know if it is affordable. Development of personalized medicine would also affect the pharmaceutical markets, which are described in detail in a special report on this topic (Jain 2015a).

## Perceived Financial Concerns

The pharmaceutical industry expects new technologies to facilitate the development and introduction of "blockbuster drugs", which are currently defined as those generating >$1 billion per year. It is common belief in the pharmaceutical industry that blockbuster drugs must target large patient populations and concern has been expressed that personalized medicine may shrink the market for a particular drug by limiting the number of those who can take it. Therefore, the pharmaceutical companies are interested in using genetics to develop drugs for the population in general and not for a particular genotype. But the important role of genetic variability in disease and therapy revealed by pharmacogenomics suggests that smaller, genetically defined patient populations can be treated more effectively. This would require a complete rethinking and retooling of the genetics-based drug discovery and development on part of the pharmaceutical industry.

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## Personalized Medicine and Orphan Drug Syndrome

There is no satisfactory definition of an orphan disease. In the USA it is defined as one that affects <200,000 individuals but this number is less in other countries with smaller populations. In general, an orphan disease is a condition that affects <1 person per 10,000 of population. Most of the orphan diseases are genetic in origin. The common factor between personalized medicine and the orphan drugs is a small or targeted patient population. There are examples of orphan drugs that are personalized medicines as well:

- Kalydeco for cystic fibrosis (CF), approved by the FDA in 2012, addresses the underlying cause of CF rather than the symptoms. It is a targeted therapy for patients with the specific G551D mutation that is found in only 4 % of CF cases in the US.
- Crizotinib (Xalkori), which targets a fusion containing ALK found in 5.5 % of non-small cell lung cancer patients.
- Vemurafinib (Zelboraf), Dabrafenib (Tafinlar), and Trametinib (Mekinist) for targeting BRAF V600E mutation found in 15 % of melanoma patients.

Segmentation of a common disease into subcategories on pharmacogenomic basis might create a small population for a certain drug – orphan drug syndrome. Orphan Drug Law in the US and similar laws in European Union, Japan and some other countries provide financial incentives for the pharmaceutical companies developing products for orphan diseases. The same could be applied to personalized medicine. Potential problems in this area, ethical and those related to cost-effectiveness, remain to be addressed.

## Commercial Aspects of Pharmacogenomics

The commercial aspects of personalized medicine that are discussed are based on considerations of the cost of various technologies that will be used in developed such medicines. Systematic pharmacoeconomic studies of pharmacogenomics have not yet been carried out. The economic benefits can be predicted on the basis of current progress made in genomics and will be a sequel of reduced time for R & D and introduction of the product into the market.

### *Cost of DNA Testing*

DNA tests for identifying an individual is simple and cheap. Commercial laboratories offer DNA testing for paternity and other relationships for as little as $130. Legal setting raises the costs. There are over DNA 1,200 tests available, mostly for diagnosis of diseases. The cost varies from $150 to over $1,000 with an average of $500. The costs are expected to drop in the future as the use increases. Markets for molecular diagnostics are described in a special report on this topic (Jain 2015b).

## *Lowering the Cost of Sequencing the Human Genome*

Previously it was very expensive to sequence the three billion base pairs of DNA found in humans. Therefore, large scale sequencing was carried out mostly at special sequencing centers and is restricted to major expensive projects. The immediate goal of the NIH's National Human Genome Research Institute (NHGRI) is to support research to lower the cost of these projects more than 100-fold in order to allow scientists to sequence genomes of human subjects involved in studies to find genes relevant for disease. The longer-term goal of NHGRI's "Revolutionary Genome Sequencing Technologies" grants totaling more than $32 million is the development of breakthrough technologies that will enable a human-sized genome to be sequenced for $1,000 so that this process can be used in routine medical tests and allow physicians to tailor diagnosis, prevention, and treatment to a patient's individual genetic makeup. In 2011, Illumina lowered the cost of its human whole-genome sequencing services to $5,000 per genome for projects of 10 samples or more, and $4,000 for projects of 50 samples or more. The services were offered through the Illumina Genome Network and compete directly with human whole-genome offerings from Complete Genomics and Life Technologies.

The first human genome sequence, completed by the federally financed Human Genome Project in 2003, cost a few hundred million dollars. In 2007, the genome sequence of James D. Watson was completed at a cost of about $1 million. In 2007, NHGRI pumped over $15 million into 12 new grants to develop methods and technologies aimed at "dramatically" reducing the cost of genomic sequencing, with a target of lowering the price of sequencing individual human genomes down to $1,000.

In 2008, the cost was ~$100,000. Knome, a company that offers to provide consumers with their DNA sequence, charged $350,000 that included not just the sequencing costs but also the analysis of the data and the customer service. In 2008, Life Technologies' latest machine could sequence a human genome for $10,000. This amount included only the cost of consumable materials, and not labor or the machinery.

Complete Genomics started charging $5,000 in 2009 for determining the sequence of the genetic code that makes up the DNA in one set of human chromosomes. Its sequencer was not that much different from rival machines, but miniaturization enabled it to use only tiny amounts of enzymes and other materials. This price represented another step toward the long-sought goal of the "$1,000 genome." At that price point it was affordable for people to obtain their entire DNA sequences, giving them information on what diseases they might be predisposed to or what drugs would work best for them. Complete Genomics did not offer a service to consumers, but provided sequencing service for consumer-oriented companies such as Knome. Most of its customers were pharmaceutical companies or research laboratories that conduct studies aimed at finding genes linked to diseases. Such studies might look at the DNA of 1,000 people with a disease and 1,000 people without the disease. Complete Genomics performed ~1,000 human genome sequences in 2009 and 20,000 in 2010, with a goal of completing a million by 2013. Volume could further drive down prices. By end of 2010, companies developing DNA sequencing and related technologies, as well as those working on sequencing-based genetic

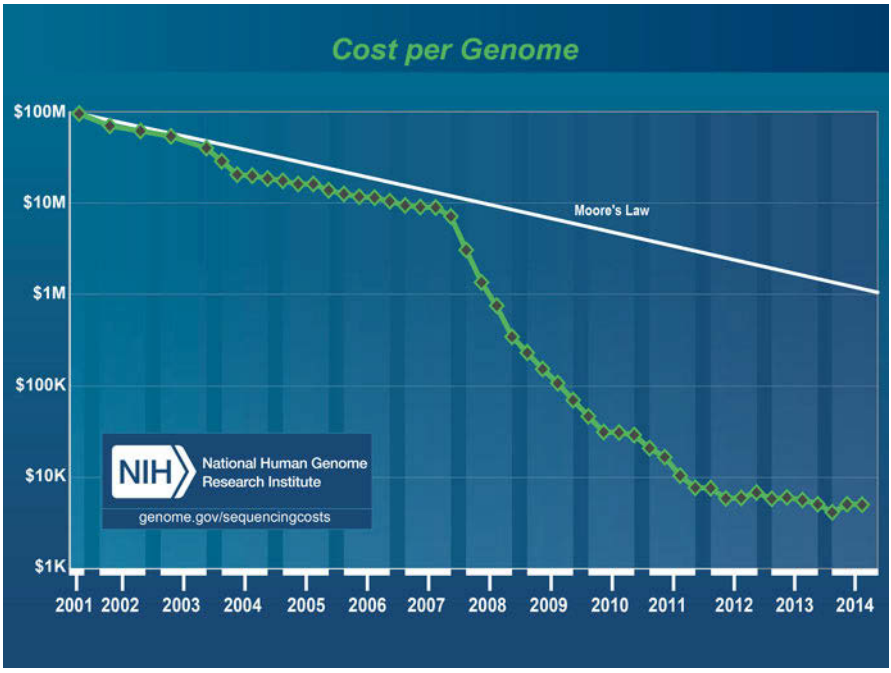


**Fig. 23.1** Cost of sequencing per genome (DNA sequencing costs: data from the NHGRI Genome Sequencing Program. http://www.genome.gov/sequencingcosts (accessed on 8 Jan 2015))

tests had received $600 million in grants and tax credits under Qualifying Therapeutic Discovery Project (QTDP) in the US. They were among almost 3,000 small biotech companies who received a total of $1 billion under the QTDP program. In 2011, Illumina lowered the cost of its human WGS services to $5,000 per genome for projects of 10 samples or more, and $4,000 for projects of 50 samples or more. The services were offered through the Illumina Genome Network and competed directly with human whole-genome offerings from Complete Genomics and Life Technologies.

In 2012, Life Technologies' Benchtop Ion Proton™ Sequencer could decode a human genome in 1 day for $1,000. A graph of the drop of cost of sequencing per genome at the NHGRI Genome Sequencing Program is shown in Fig. 23.1.

## *Cost of Genotyping*

Currently, it typically costs a drug company about $1 billion to develop, test, and bring to market a single drug. Pharmacogenomic data could hasten clinical drug trials, allowing researchers to design and conduct safer, more targeted trials on a

particular drug. The results of such a trial would be far more conclusive and focused than those of trials that do not use pharmacogenomic data. By reducing both the time of drug development, the number of patients required and the failed clinical trials, pharmacogenomics is expected to reduce the cost of drug development. The question now is the cost of genotyping.

Genome-wide association studies require at least 100,000 SNPs to be genotyped in, for example, 500 cases and 500 controls. This represents 100,000,000 genotypes for each analysis. Using today's technology, an amplification method is required, whether it is on an individual SNP basis using PCR or by whole genome amplification. A rapid discrimination mechanism to determine the genotype of each sample and some way of rapidly reading out and capturing the data are required. Many technologies are being developed to solve these practical issues, but they invariably require a PCR step. The miniaturization of PCR using microfluidics may provide an opportunity to reduce costs, as well as multiplexing both the amplification steps and the detection steps. Nanotechnology with nanopore DNA sequencing and single molecule detection is another promising approach.

Another problem associated with the whole genome scans in humans is that the technology platform will have to deliver between 250,000–1,000,000 genotypes a day to make the time frame for these studies reasonable. Current cost ranges between 10¢ and $1 per genotype. For example using TaqMan technology, 1,000,000 genotypes would cost $1 million ($1 per genotype) or oligo ligation assay and ABI 377 technology would cost $500,000 (50¢ per genotype). Even at the level of the individual patient, to genotype 300,000 SNPs is an expensive proposition. To enable such approaches to be used widely the cost per genotype has to come down from the current cost to 1¢ per genotype. Current genotyping arrays can reveal most of the common SNPs for $1,000 and it remains to be seen as to how much more meaningful information whole-genome sequencing can add to that, even though the goal of $1,000 genome has been reached.

## *Cost of Pharmacogenomics-Based Clinical Trials*

The pharmaceutical companies would, therefore, have a better understanding of the cost required to complete the development of the drug and the likely economic return on their investment before proceeding to a phase III clinical trial. The cost for pharmacogenomics-based clinical trials would be less than that of conventional clinical trials because fewer patients would be required for such trials. If 5,000 patients are required for current clinical trials, use of pharmacogenomics should enable all the three phases to be completed with less than 2,500 patients – a saving of more than 50 %. In addition, understanding the correlation between drug response and genomic differences would enable pharmaceutical companies to improve the marketing of their drugs by identifying those patients for whom particular drugs are likely to be most effective. Several pharmaceutical companies are now using genotyping in most of their clinical trials while others are not.

## *Personalized Medicine and the Rising Healthcare Costs in the US*

Overall, health care inflation continues to rise precipitously. The total health care expenditures in the US in 2014 were >$3.8 trillion. Hospital care, physician services, and prescription drugs account for most of this spending. The health care system in the US is in need of a new paradigm to reduce spending. Personalized medicine provides an invigorating solution for lowering the cost of health care.

It is generally recognized that drugs are the cheapest and least traumatic way of dealing with chronic illnesses. Proliferation of surgical procedures and hospitalization has raised the costs of healthcare. Refinement of surgical procedures to become minimally invasive and use of products of biotechnology to improve the results are some of the advances in surgery. Most of the surgical procedures for peptic ulcer have become obsolete by the introduction of rational anti-ulcer drugs. It is likely that essential surgery of the future will be limited to trauma, emergencies such as hemorrhages, anatomical corrections of pathology, organ transplants (where medical therapies have failed), implantation of electronic devices, removal of benign tumors, cancer of some organs etc. Surgery will have only a subsidiary role for cancer of organs such as brain for which more effective non-surgical therapies such as gene therapy would be developed.

Currently <15 % of the world's healthcare budget is spent on drugs. It is likely to increase during the next decade, depending upon what new and effective medicines emerge from the pipelines of biopharmaceutical companies. Many of the currently incurable diseases such as Alzheimer's disease will have rational therapies during the next decade. Although introduction of treatments for incurable diseases would raise the drug costs, it will reduce the total cost of healthcare such as on nursing home care and other palliative drugs, which would no longer be necessary. However, simple introduction of new medicines to the population in general may involve waste of money as some patients may not respond to these. Here, the importance of personalized medicines based on pharmacogenomics becomes obvious. These may be more expensive to develop and may cost more, but will eventually lower the healthcare costs.

There are individual examples of high cost of personalized drugs of rare diseases. One example quoted by those concerned with high cost of personalized medicine is cystic fibrosis (CF) drug Kalydeco, which may help patients with certain CF gene mutations, but costs $300,000 a year. Other non-personalized biopharmaceuticals for some rare orphan diseases are also extremely expensive. No field study has been done so far to determine the overall cost of healthcare based on personalized medicine. However, overall cost of healthcare is expected to decrease with personalization due to following reasons:

- Increased efficacy of personalized medicines will offset the higher prices of drugs
- Increased safety of personalized medicines will reduce costs due to adverse reactions to conventional drugs

- Reduction of high expense of hospital stay
- Predictive medicine will reduce costs by prevention

The healthcare reform bill in the US, which passed and is being implemented, is unlikely to reduce healthcare costs as claimed. There is no mention of the role of personalized medicine in it.

## *Genetic Testing and Cost of Healthcare*

One concern surrounding increased and widespread genetic testing is that it could lead to increased use of an already strained healthcare system in the US. However, according to a recent study multiplex genetic testing may not lead to increased use of healthcare services (Reid et al. 2012). As part of the Multiplex Initiative, a NIH-funded, multidisciplinary research effort to examine how the general public views genetic testing, participants were tested for 15 risk variants for a number of common diseases such as type 2 diabetes, coronary heart disease, and melanoma, etc. Persons offered and completing multiplex genetic susceptibility testing used more physician visits before testing, but testing was not associated with subsequent changes in use. This study supports the supposition that multiplex genetic testing offers can be provided directly to the patients in such a way that use of health services is not inappropriately increased.

## *Reducing Healthcare Costs by Combining Diagnostics with Therapeutics*

Cost-effective diagnostics are but a prelude to an era of cost-effective personalized medicine. The real potential is in better targeting expensive drugs to those who will benefit from them, thereby both cutting wasteful expenditure and decreasing adverse events associated with treating non-responders. Some examples of this are as follows.

The anticancer drug Avastin (Genentech/Roche) costs $50,000–$100,000 per year of treatment but works in fewer than 50 % of patients. Avastin is an approved therapy for lung cancer, kidney cancer, colorectal cancer and brain cancer, but its approval for HER2-negative metastatic breast cancer was withdrawn by the FDA in December 2010 because of lack of efficacy as well as adverse effects. Avastin may be useful in a targeted group of breast cancer patients but there is no available test that can identify such patients. Given that Avastin may generate $12 billion in peak sales, the low rate of efficacy translates into billions of dollars in misdirected healthcare spending. A test for Avastin response, such as that in development by BG Medicine, could save the healthcare system as much as $6 billion per year if all nonresponders could be removed from the treatment pool. Assuming that a test of this sort is introduced at the beginning of 2015 and is 100 % adopted, cumulative savings of $40 billion could be realized by 2020.

Oncotype Dx (Genomic Health) is a test with compelling cost-saving potential. It is used to predict chemotherapy benefit for patients who have node-negative, estrogen receptor positive (node-, ER+) breast cancer. By averting unnecessary chemotherapy, the test has been shown to save about $2,000 per patient. Extending this cost savings to the roughly 100,000 new cases of node-, ER+ breast cancer in the US each year, this test could save the US healthcare system up to ~$200 million a year or about $2 billion over the 10-year time horizon under legislative consideration for the healthcare reform bill.

Allomap (XDx) is a noninvasive test that is used instead of biopsy in the management of heart transplant patients after surgery. The total potential cost savings is estimated at roughly $20 million per year (about $12 million for payers as well as ~$8 million for hospitals and transplant centers). This scenario, in which a drug with high sales but low efficacy is targeted by diagnostics companies, may become a pattern in the near future, multiplying cost savings.

## *Costs of Pharmacogenetic Testing*

Among direct to consumer personal genetic testing companies, the cost of 23andMe's service, at $399, is the least expensive. Navigenics' SNP-genotyping service (acquired by Life Technologies in 2012), which uses Affymetrix arrays, costs $2,500, while Decode Genetics' program, which uses Illumina's Human 1 M BeadChips, costs $985. New Hope Medical, a clinic that provides diagnostics and therapies not readily available in conventional medicine, charges between $475 and $900 for genomic testing for 12 and 25 SNPs respectively linked to certain conditions. Meantime, a full-genome scan by Knome costs at least $350,000.

## *Pharmacogenetics to Reduce the Cost Incurred by Adverse Drug Reactions*

Increase in treatment efficacy by individualize treatment is difficult to measure in financial terms but the savings from reduction of adverse reactions would be considerable. Over two million adverse drug events due to medicines experienced by patients in the US, or admissions to hospital because of an adverse reaction are estimated to cost over $100 billion per year to the health-care industry. A study in France found that adverse reactions to drugs accounted for 3.5 % of admissions to a cancer institute and 1.8 % of the hospital budget. Even if personalized medicine reduces adverse reactions by a small percentage, the resulting savings to the healthcare industry would be considerable.

Genetic polymorphisms of the drug-metabolizing cytochrome P-450 (CYP) enzymes CYP2C9, CYP2C19 and CYP2D6 have been characterized. This is of clinical importance mainly in patients having two non-functional alleles, phenotypically

characterized as "poor metabolizers" (1–10 % of Caucasians). Studies have shown that pharmacogenetic analyses will significantly contribute to reducing treatment costs for drug-induced adverse reactions and costs of sick leave, by predicting the best drug and the most effective and safest dosage. The expenses of full genotyping (CYP2C9/2C19/2D6) are less than financial loss from 1 day of sick leave of an employee. The question has been raised: are pharmacogenetic analyses coming to the point where they drive down costs incurred by illness?

CYP450 genotyping has potential to improve efficacy of 10–20 % of all drug therapy and reduce incidence of ADRs by 10–15 %. CYP2D6 genotyping shows mutations causing ultra-rapid metabolism leading to hugely increased levels of active compounds such as codeine, which can cause symptoms of overdosage with usually recommended doses. According to a study by Roche, its product AmpliChip CYP450 could cut costs in 44 % of cases. Considering the current rate of growth, the US health care system could potentially save $21 billion by 2020.

## *Cost Effectiveness of HIV Genotyping in Treatment of AIDS*

Costs of antiretroviral therapy for HIV-infected patients have increased at a time when most countries are attempting to contain health care costs. Part of this increase results from HIV drug resistance and subsequent shift to more complex and costly therapies. Genotypic guided treatment is associated with better virologic outcome. However, it is not yet known whether it will be cost-effective. Two examples show the cost-effectiveness of HIV genotyping.

VIRADAPT study, a prospective, open-label, randomized trial compared patients assigned to standard of care versus genotypic guided treatment for 6 months. Total follow-up for the extended trial was 1 year. Costs were computed from the viewpoint of the health care system in France. Genotyping using TruGene HIV-1 assay, estimated at $500 per test, resulted in yearly costs per patient of >$20,000 in the standard of care group and >$18,000 in the genotyping group. Drug costs represented 55 % of total costs. There was a trend toward a decrease in drug costs in the genotyping arm, the greatest reduction being in the decreased use of protease inhibitors in the genotyping arm. The additional expense of genotyping appeared to be offset by the savings obtained in drug costs.

HIV genotyping with secondary resistance testing increases life expectancy of AIDS patients by 3 months, at a cost of ~$18,000 per quality-adjusted life-year (QALY) gained. The cost-effectiveness of primary resistance testing is $22,000 per QALY gained with a 20 % prevalence of primary resistance but increases to $70,000 per QALY gained with 4 % prevalence. The cost-effectiveness ratio for secondary resistance testing remains under $25,000 per QALY gained, even when effectiveness and cost of testing and antiretroviral therapy, quality-of-life weights, and discount rate are varied. It is concluded that genotypic antiretroviral resistance testing following antiretroviral failure is cost-effective. Primary resistance testing also seems to be reasonably cost-effective and will become more so as the prevalence of primary resistance increases.

### *Cost-Effectiveness of Warfarin Pharmacogenomics*

Review of studies incorporating clinical efficacy data of genotype-guided dosing algorithm had shown that warfarin pharmacogenomics would improve quality-adjusted life-years gained (You 2011). However, it is unlikely to be cost-effective for patients in general. Important factors for improving the cost-effectiveness include low genotyping cost, high effectiveness in improving anticoagulation control and lowering adverse events. Application of warfarin pharmacogenomics could possibly be cost-effective in selected patient groups with high bleeding risk or practice sites with suboptimal management of anticoagulation control.

### *Cost-Benefit Analysis of KRAS and BRAF Screening in CRC*

According to a study screening for KRAS and BRAF mutations can reduce the cost of anti-EGFR treatment for metastatic CRC but with a very small reduction in overall survival (Behl et al. 2012). Metastatic CRC patients whose tumors harbor mutations in KRAS (and to a lesser extent, in BRAF) are unlikely to respond to costly anti-EGFR therapies. Screening of patients who are candidates for these therapies for mutations in one of these genes (KRAS) has been recommended, with the goal of providing treatment to those who are likely to benefit from it while avoiding unnecessary costs and harm to those who are not likely to benefit. However, the real-world impact of mutation screening for both KRAS and BRAF is unclear. The researchers found that compared with no anti-EGFR therapy, screening for both KRAS and BRAF mutations showed a very high incremental cost-effectiveness ratio, i.e. it was very costly in relation to its benefits. Compared with anti-EGFR therapy without screening, screening for KRAS mutations saved approximately $7,500 per patient; adding BRAF mutation screening saved another $1,023, with little reduction in expected survival. In general, these results are less supportive of the use of anti-EGFR therapy than previous analyses, and they indicate lower cost savings from KRAS testing than previously reported. Although it cannot be confirmed that anti-EGFR therapy is a cost-effective use of health care resources, the results affirm that KRAS testing is cost-saving and BRAF testing may offer additional savings.

Molecular testing is as much about generating cost savings by identifying nonresponders as it is about improving survival by identifying responders, and that good modeling must account for the fact that community practice (as opposed to clinical trials) is messy. This study of an unusually accurate test raises important issues that should be considered for other molecular tests in other settings.

### *Lowering the High Costs of Cancer Chemotherapy*

Pharmacogenomics for cancer is being driven by the fact that treatment costs are so high and getting higher. Molecular biomarkers will enable us to decide who really needs expensive therapy. The costs will be reduced significantly as more genetic variants come into play, which are important in terms of drug response. There might be gene chips that are specifically tailored toward different types of therapy, and one could look at many different genotypes at the same time in a single patient sample. So costs should go down as discoveries are made.

Another contributor to high costs of care of cancer patients are adverse effects from chemotherapy. Identification of patients who might react adversely to a treatment could help in saving costs by avoiding administration of drugs to patients at risk of adverse reactions. Researchers are looking at sensitivity to chemotherapies within families and identifying candidate genes that contribute to susceptibility to anticancer drug toxicity. Studies of cell lines from CEPH (Centre d'Etude du Polymorphisme Humain, France) families have shown that susceptibility to the toxic effects of the anticancer drug cisplatin is significantly heritable. CEPH collects biological samples from large families which serve as reference families for genetic research. With the help of gene expression profiling, it is possible to identify the genes responsible for conferring drug susceptibility. A clinical trial by researchers at the University of Chicago has demonstrated the predictive significance of genotyping for variants that affect drug pharmacodynamics. They genotyped 20 patients, looking for variations in the promoter that controls activity of the enzyme UGT1A1, which is important for detoxification of the active metabolite of irinotecan, an effective anticancer drug that can cause diarrhea and neutropenia. One UGT1A1 variant contains a TA repeat of the TATA sequence in the promoter. The toxic effects were found only in patients who possessed at least one allele of that polymorphism.

## Concluding Remarks on the Economics of Personalized Medicine

Several studies point to benefits of personalized medicine by improving efficacy and safety. From an ethical point of view, a physician is required to recommend the best available treatment. However, with rising cost of healthcare in advanced countries such as the US and poverty in some developing countries struggling to provide basic healthcare, there are problems in implementation of personalized therapies, some of which cost more than conventional medicines. The pharmaceutical industry is adapting to development of trend in personalized medicine, but some controversies need to be resolved. Although case studies of application of personalized medicine have shown benefit for patients and cost-effectiveness, the barrier to large-scale real-world adoption of this approach requires a change in health policy.

At point-of-care, the case studies personalized medicine will need to measure outcomes, which are important for policy-makers, as evidence of clinical utility (van Rooij et al. 2012). There is considerable discussion on issues related to comparative effectiveness of personalized medicine, drug development, and payer approaches to evaluation as well as reimbursement of pharmacodiagnostics in the US and Europe (O'Donnell 2013).

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# Chapter 24
# Future of Personalized Medicine

## Introduction

Several studies of the human genome are still going on and some are planned. A selection is described briefly in the following pages.

## Ongoing Studies

### *Personal Genome Project*

Achieving personalized medicine will require extensive research on highly re-identifiable, integrated datasets of genomic and health information. A Personal Genome Project (PGP) was launched as a sequel of the Human Genome project and volunteers were recruited to make their own genomic and phenomic data available. Participants in the PGP choose to forgo privacy via institutional review board-approved "open consent" process. These resources were planned to include full (46-chromosome) genome sequences, digital medical records and other medical information that would become a part of personal health profile. It also includes comprehensive data about RNA and protein, body and facial measurements and imaging such as MRI. Human cell lines representing each subject are deposited in a repository at the National Institute of Genome Medical Sciences. Details of PGP can be found at the following web site: http://www.personalgenomes.org/.

The findings after enrollment of more than 1,800 participants, including WGS of 10 pilot participant genomes (the PGP-10), have been published (Ball et al. 2012). The Genome-Environment-Trait Evidence (GET-Evidence) system, which automatically processes genomes and prioritizes both published and novel variants for interpretation, was introduced. In the process of reviewing the presumed healthy PGP-10 genomes, the authors found numerous literature references implying serious disease. Although it is sometimes impossible to rule out a late-onset effect,

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stringent evidence requirements can address the high rate of incidental findings. To that end the team developed a peer production system for recording and organizing variant evaluations according to standard evidence guidelines, creating a public forum for reaching consensus on interpretation of clinically relevant variants. Genome analysis becomes a two-step process: using a prioritized list to record variant evaluations, then automatically sorting reviewed variants using these annotations. Genome data, health and trait information, participant samples, and variant interpretations are all shared in the public domain. There is an open invitation to others to review the results using participant samples and contribute to interpretations. This public resource and methods are offered to further personalized medical research. In the ongoing project, the organizers hope to enroll 100,000 participants.

## *Genome-Wide Association Studies*

The NIH is seeking public input on a proposed new policy designed to facilitate the research community's access to data resulting from NIH-funded, genome-wide association studies (GWAS), which would lead to the development of a centralized NIH data repository. NIH published a "Request for Information in the Federal Register" in 2006. GWAS rely on the newly available research tools and technologies to rapidly and cost-effectively analyze genetic differences between people with specific illnesses, such as diabetes or heart disease, compared to healthy individuals. The differences may point to genetic risk factors for the development or progression of disease. Several NIH institutes recently launched, or are planning, GWAS initiatives with the expectation that the results will accelerate the development of better diagnostic tools and the design of new, safe and highly effective treatments. This will be an important contribution to genomics-based health care and personalized medicine.

As numerous GWAS programs get underway, NIH seeks to harmonize the policies by which the results will be made available to researchers. The proposed GWAS Policy calls-on NIH-funded GWAS investigators to quickly submit genetic data (genotypes) along with relevant health information (phenotypes) about individuals to a centralized NIH data repository. Data will be submitted in a form that protects the privacy and confidentiality of research participants. The data will be made freely available to all approved researchers to accelerate their studies. The draft policy also proposes terms and conditions for investigators to access GWAS data for research purposes. Data will be released in a manner that preserves the privacy and confidentiality of research participants.

NIH encourages patenting of intellectual property that addresses public need, such as creating new treatments that can be brought to the clinic, but seeks to prevent premature or inappropriate patents that impede future research. Because publication credit is critical to academic promotion, the proposed NIH policy also defines a grace period during which GWAS data will be available for access, but principal investigators submitting the data would be the only ones allowed to publish analyses

in scientific journals. The policy also asks that recipients of GWAS data acknowledge the submitting investigator in any published works.

The NIH set aside $6 million in funding from 2007 to 2009 to support the development of methods for identifying gene-environment interactions in genome-wide association studies. According to a request for applications NIH issued in 2006 titled, "Methods of Analysis of Gene-Environment Interactions in Complex Diseases: The Genes and Environment Initiative," NIH awarded five grants at up to $400,000 in total costs per year per award amounting to $2 million in fiscal year 2007 for the program, which falls under the broader Genes and Environment Initiative, a 4-year, NIH-wide program was proposed in the 2007 budget and is still awaiting US Congressional approval. NIH is seeking applicants who will "develop and test innovative, informative, and cost-effective methods and analytical strategies for identifying gene-environment interactions in genome-wide association studies, sequencing studies, linkage analyses, or candidate gene approaches with broad applicability in complex diseases." Examples of approaches are:

- Analytical methods that model combinations of SNPs and environmental exposures to detect nonlinear interactions.
- Analytical methods that incorporate environmental covariates in genotype-to-phenotype mapping relationships.
- Algorithms and strategies to evaluate non-genetic factors on phenotypes of complex diseases and test associations between SNPs or haplotypes and phenotypes.
- Novel approaches to analyze findings from pharmacogenomic studies.

## *The 1000 Genomes Project*

The 1000 Genomes Project (http://www.1000genomes.org/) is being carried out by an international consortium including the Wellcome Trust's Sanger Institute in the UK, the US National Human Genome Research Institute, and the Beijing Genomics Institute in China. The estimated cost is $30–$50 million. A thousand persons will have their genomes sequenced in an ambitious 3-year project that will create the most comprehensive catalogue so far of human genetic variation. These volunteers have already been recruited from Africa, Asia, America, and Europe. They have given informed consent for their DNA to be analyzed and placed in public databases. The donors are anonymous and will not have any of their medical information collected because the project is developing a basic resource to provide information on genetic variation. The goal of the 1000 Genomes Project is to uncover the genetic variants that are present at a frequency of 1 % or more in the human genome. Three 1000 Genomes pilot projects, which began in 2008 aim to achieve low coverage of 180 individuals, high coverage of two parent-offspring trios, and targeted sequencing of 1,000 genes in approximately 1,000 individuals, are nearing completion. Those efforts seem to be generating high-quality data and have already uncovered new genetic variants. So far, the 1000 Genomes Project has

generated 3.8 terabases of data. In 2009, the project is expected to up that dramatically, producing a petabyte of data.

Beyond the direct implications for the 1000 Genomes Project, the effort has spurred researchers to pioneer and evaluate methods that benefit other research efforts as well. For example, researchers have been working with high-throughput sequencing, developed new approaches for exchanging and analyzing data, discovering SNPs and CNVs, and making imputations based on next-generation sequence data. There a need, however, for developing shared data formats for different stages of the analysis. In the absence of standard formats or a clear framework for such analysis, efforts to decipher the genetic information would be delayed. Consequently, team members are working to develop draft formats to aid this analysis.

## *Genomics of Aging in a Genetically Homogeneous Population*

According to UNESCO's Preservation of Parsi Zoroastrian Project, 31 % of the Parsi population in India lives beyond the age of 60, compared to 7 % nationally (http://theavestagenomeproject.org/). A better understanding of the genetic causes of longevity could have a major impact on the Indian Government's healthcare budget and drug companies' marketing efforts. Affymetrix signed an agreement with Avesthagen Ltd (Bangalore, India), whereby Affymetrix' microarray technology will be used for the AVESTAGENOME Project™, which will explore the genetic basis of longevity and create a genetic, genealogic and medical database of the Parsi-Zoroastrian population. The use of Affymetrix technology will enable researchers to correlate genes with longevity, as well as neurodegenerative conditions, breast cancer, diabetes and other complex diseases that affect the Parsi community. The Parsi community was selected because of its longevity and its relatively genetically homogeneous population. This project takes a systems biology approach that encompasses not only genotyping but also expression profiling and transcriptomics. The genotyping phase of the project, which began in 2007, consisted of 10,000 samples in the first year. By the middle of 2008, the team had performed expression profiling and transcript mapping experiments across a subset of the samples.. Genetic information for The AVESTAGENOME Project™ is being collected following informed consent. Data confidentiality is being maintained as in accordance with the Indian Council of Medical Research guidelines.

## *Translational Science and Personalized Medicine*

Translational science deals with transfer of technologies from preclinical research into clinical application. Table 24.1 shows translational methods that are relevant to personalized medicine. Biomarkers play an important role as described earlier.

**Table 24.1** Methods of translational science that are relevant to personalized medicine

| |
|---|
| **Biomarkers** |
| Biomarker discovery and development, e.g. imaging or serum |
| Biomarker scoring systems to grade their predictive potency |
| Translational toxicology using biomarkers |
| **Preclinical to clinical studies** |
| Animal models that are representative of human disease |
| Cautious transfer of results of preclinical studies to predict clinical effects |
| Careful early human exploratory clinical trial design prior to phase I/II trials |
| Following a consistent set of biomarkers from preclinical studies to phase III trials |
| Image analysis software should be the same for preclinical and clinical studies |
| **Bioinformatics** |
| **Human genetics** |
| **Systems biology approaches** |



## *Translation of Genomic Research into Genetic Testing for Healthcare*

Advances in genomics have led to mounting expectations in regard to their impact on health care and disease prevention. There is a need for a comprehensive research agenda to move human genome discoveries into health practice in a way that maximizes health benefits and minimizes harm to individuals and populations. A framework has been presented for the continuum of multidisciplinary translation research that builds on previous characterization efforts in genomics and other areas in health care and prevention (Khoury et al. 2007). The continuum includes four phases of translation research that revolve around the development of evidence-based guidelines:

- Phase 1 translation (T1) research seeks to move a basic genome-based discovery into a candidate health application (e.g., genetic test/intervention).
- Phase 2 translation (T2) research assesses the value of a genomic application for health practice leading to the development of evidence-based guidelines.
- Phase 3 translation (T3) research attempts to move evidence-based guidelines into health practice, through delivery, dissemination, and diffusion research.
- Phase 4 translation (T4) research seeks to evaluate the "real world" health outcomes of a genomic application in practice.

Because the development of evidence-based guidelines is a moving target, the types of translation research can overlap and provide feedback loops to allow integration of new knowledge. Although it is difficult to quantify genomics research is T1, no more than 3 % of published research focuses on T2 and beyond. Evidence-based guidelines and T3 and T4 research are scarce. With continued advances in genomic applications, however, the full continuum of translation research needs adequate support to realize the promise of genomics for human health.

## *Long-Term Behavioral Effects of Personal Genetic Testing*

In 2008, Scripps Translational Science Institute (STSI), Navigenics (now acquired by Life Technologies), Affymetrix, and Microsoft embarked on a decades-long study to determine the long-term behavioral effects of personal genetic testing. Genetic scans will be offered to up to 10,000 Scripps Health system employees, family members, and friends in the study, the first of its kind, said STSI. Eventually, researchers hope to determine whether participating in personal genomic testing spurs individuals to make beneficial lifestyle changes such as improving their diet and exercise regimes. The team plans to track participants' lifestyle changes using self-reported health questionnaires. Participants will complete the questionnaires at baseline and again 3 and 6 months after receiving the personal genetic test, which is designed to assess each individuals' genetic propensity for more than 20 health conditions, including diabetes, hearts disease, and some cancers. Those enrolled will also be asked to participate in surveys periodically over the next 20 years. The results will be compiled in a database hosted by the Scripps Genomic Medicine program. To maintain participants' genetic privacy, researchers will de-identify both saliva samples and health assessment questionnaires, encrypt the data, and store it in a secure database. In addition, researchers plan to use genetic variations identified in the study to improve their understanding of the genetics underlying diseases and the application of this genetic information for preventing, diagnosing, and treating diseases. Affymetrix will perform the genome scans, while Navigenics (now acquired by Life Technologies) will interpret the results and offer guidance on steps individuals can take to try to decrease health risks based on their personal genetic information.

## *Personalized Predictive Medicine*

There has been an increasing emphasis on preventive medicine during the past decade and now predictive medicine is gaining popularity as an approach to improve healthcare in the future. Companies involved in predictive healthcare. Predictive medicine involves prediction of risk of disease in an individual and its personalized management. It is sometimes referred to as preemptive approach as it involves treatment before the disease develops. By the time most diseases are diagnosed, some damage is already done and in some situations it is irreparable. Moreover, chances cure of diseases such cancer would be anticipated to improve with this approach. Advances in molecular diagnostics, proteomics, and metabolomics are facilitating the development of tests for predictive medicine. The concept of predictive medicine is extended further to predict response of the disease to a particular therapeutic. A significant reduction in disease-related mortality as well as a reduction in costs can be expected if prevention and screening are focused on individuals at risk. In the pharmaceutical industry, predictive modeling of disease can be used to test efficacy of drugs before developing them.

### *Connected Health and Personalized Medicine*

The term 'connected health (CH)' has been increasingly used in recent years to describe this new technology enabled model of healthcare delivery. The following definition proposed: 'Connected Health encompasses terms such as wireless, digital, electronic, mobile, and tele-health and refers to a conceptual model for health management where devices, services or interventions are designed around the patient's needs, and health related data is shared, in such a way that the patient can receive care in the most proactive and efficient manner possible' (Caulfield and Donnelly 2013).

Over the last decade, connected health (CH) has shown great value in the management of chronic disease (CD), but has limited application in preventing these diseases that remain a huge burden to the society. Technological advances have made determination of genetic predisposition to disease possible and have gained wide use in medicine of developing personalized medicine. There is growing interest in the application of these genetic tests in predicting risk for complex genetic diseases as direct-to-consumer tests are increasingly becoming available and affordable. CH has shown great potential in collecting phenotypic data, which can be integrated with genomic data to deliver a more precise and personalized preventive care for patients. The goal of a CH program that uses genetic data would be to monitor individuals' risk factors and predict the onset of CD, which would be coupled with advice to prevent the onset of disease. However, the challenge is that many CDs are due to complex interaction between genes and modifiable environmental risk factors that are still under-studied (Agboola et al. 2013).

## Drivers for the Development of Personalized Medicine

Table 24.2 lists drivers for the development of personalized medicine in the next decade.

### *Evolution of Medicine as a Driver for Personalized Therapy Markets*

There are no revolutions in medicine but evolution. This process has already been set in motion by the advent of the genomic era and will continue. The developments as shown in Fig. 24.1 will act as drivers for the markets.

**Table 24.2** Drivers for the development of personalized medicine

| |
|---|
| **Political and socio-economic drivers** |
| Public pressure on the government for safer and more effective treatments |
| Pressure from the regulatory agencies on the pharmaceutical industry to reduce adverse effects of drugs |
| Push from the insurance industry to make genetic screening more widespread |
| Threat of malpractice may pressure physicians to use genetic tests and personalized therapies |
| Political pressures to reduce cost of health care by reduction of wastage on ineffective drug therapy and care of patients with adverse reactions to drugs |
| **Scientific drivers** |
| Availability of genomic knowledge from sequencing of the human genome and developments of proteomics in the post-genomic era |
| Availability of new technologies that enable development of personalized medicine: biochips, bioinformatics, and molecular diagnostics |
| Retirement of physicians educated in the pre-biotechnology era and increasing awareness of pharmacogenomics, pharmacogenetics and molecular medicine among the younger generation of physicians |
| Introduction of personalized medicine in the academic medical centers |
| **Industrial drivers** |
| Proliferation of biotechnology companies interested in personalized medicine |
| Advances in molecular diagnostic technologies that can be applied in personalized medicine |
| Increase in the number of companies combining diagnostics with therapeutics |
| Major pharmaceutical companies developing personalized medicine |



## Collaboration Between the Industry and the Academia

The industry has taken an initiative in developing personalized medicine but collaboration with the academic basic scientists and healthcare professionals will facilitate its application. Pharmacogenetics is increasingly driven by industrial researchers, partly because of their ready access to clinical trial data on which pharmacogenetic research can be carried out. Few academic groups can afford to do so. Teaching institutions can play an important role in collecting patient data and DNA samples in clinical trials and organizing the results of their findings in databases with help of the commercial bioinformatic tools developed by the companies. The future generation of physicians in training should be learning about personalized medicine at their formative stage and the current restrictions about the participation of the commercial sector in this effort needs to be relaxed.

The industry can maintain its lead in the use of modern communication tools, such as the Internet, to allow patients to provide samples for future research yet retain control of them in the light of future developments. Both industry and academic researchers have a common goal in that both want to bring innovative solutions into clinical practice to improve health care. There is no reason why the collaboration should not be a success.

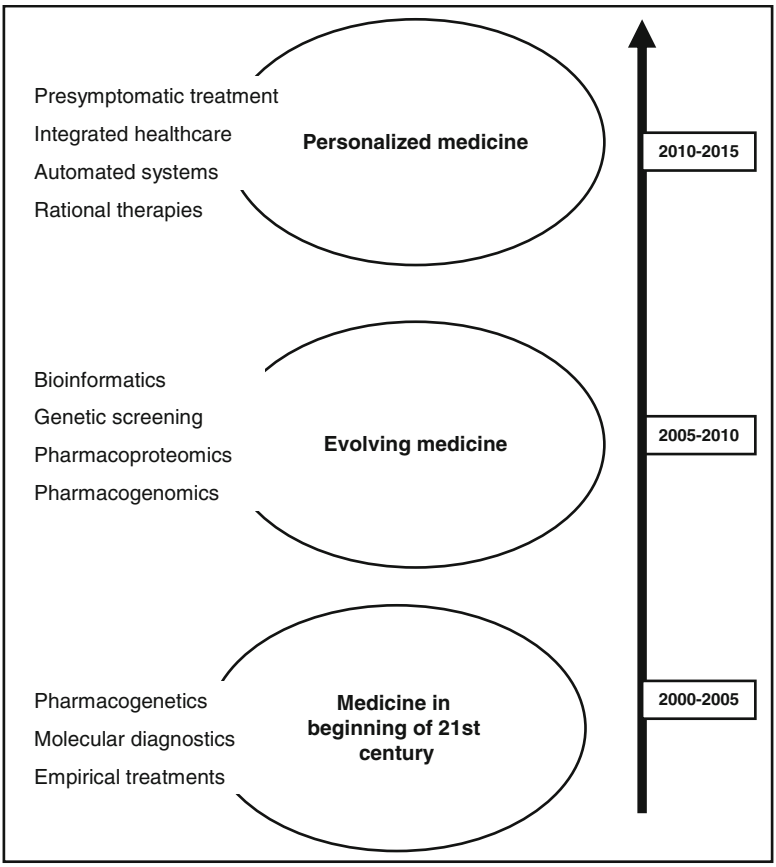




**Fig. 24.1** Evolution of personalized medicine as a market driver

## Opportunities and Challenges of Personalized Medicine

### *Prospects and Limitations of Genetic Testing*

Genotyping will be for twenty-first century medicine what the X-rays were for twentieth century clinical practice. Currently there are some reservations about the value of genetic testing in prediction of disease as there are multiple factors involved. It is currently being debated if it is worthwhile to continue with the multi-million dollar genomewide studies or to decode the entire genomes of individual patients. Although genomewide association studies have worked better and faster than expected, they have not explained as much of the genetic component of many

diseases and conditions as was anticipated, and suggestion has been made to turn more sharply toward the study of rare variants (Goldstein et al. 2014). Thus schizophrenia would be caused by combinations of 1,000 rare genetic variants, not of 10 common genetic variants. One should be concerned about diseases for which testing shows that an individual's risk is three times as great as average, but not for trivial increases in risk. The undiscovered share of genetic risk for common diseases probably lies not with rare variants, but in unexpected biological mechanisms. Also the same genetic variant carries risks that differ depending on whether it is inherited from the mother or the father.

Strong evidence suggests that rare mutations of several genes are responsible for a substantial portion of complex human disease. Evolutionary forces generate vast genetic heterogeneity in human illness by introducing many new variants in each generation. Many of them may stem from factors other than a true association with disease risk (McClellan and King 2010). Current sequencing technologies offer the possibility of finding rare disease-causing mutations and the genes that harbor them.

Genetic testing will eventually improve predictions about what diseases we are predisposed to, the timing of their onset, their extent and eventual severity as well as which treatments or medications are likely to be efficacious or deadly. Genotyping, however, does not necessarily correlate with response to medications and other factors such as environmental have to be taken into consideration in personalizing treatment. Finally, all diseases do not require personalized treatment.

Pharmacogenomics and pharmacogenetics are providing the basis for the development of molecular diagnostics to improve drug selection, identify optimal dosing, maximize drug efficacy or minimize the risk of toxicity. Rapid advances in basic research have identified many opportunities for the development of personalized treatments for individuals and/or subsets of patients defined by genetic and/or genomic tests. However, the integration of these tests into routine clinical practice remains a major multidisciplinary challenge. Although physicians and patients are optimistic about the health benefits that genetic testing might provide, neither group is well informed, and there are likely too few experts available to meet growing demands for genetic testing. Attempts to integrate genomic medicine into clinical practice are still in the early stages, and as a result, many questions surround the current state of this translation. Researchers from RAND Corporation (Santa Monica, CA), based on a review of published studies relevant to personalized medicine, concluded that many gaps in knowledge about organization, clinician, and patient needs must be filled to translate basic and clinical science advances in genomics of common chronic diseases into practice (Scheuner et al. 2008). There is a need for a large-scale effort to educate both health professionals and the public about genomic medicine, and to develop and evaluate new ways to deliver genetic services.

Genomics-based molecular profiling and related technologies may impact on the delivery of healthcare even before genomics-based drugs hit the market. Identification of genetic factors affecting the prognosis of disease is likely to be of most clinical relevance. Relationships of known genes, such as BRCA1 and BRCA2, with risk factors will be clarified; permitting evidence based preventive action in people at

high genetic risk and better quantification of risk in family members. Greatest progress will be made in understanding the genetic contribution to the intermediate phenotypes linking genes and disease, and thus the biology of the disorder, as in atherosclerotic disease. The greatest impact of personalized medicine will be in the treatment of cancer, cardiovascular diseases, infections and neurological disorders.

The emerging fields of metabonomics (metabolite profiling to identify genotype-phenotype associations) and phenomics might offer solutions for anticipating and decreasing risk of adverse drug reactions in each individual patient, but tests based on these approaches are not expected to become generally available to the practicing clinician for at least the next 5 years.

## *Genetic Testing and Concerns About Equality of Healthcare*

There is a concern genomics and associated technologies may exacerbate disparities at multiple levels because of unequal application among human populations. Unequal treatment of minority patients in the US has been attributed to many factors including poverty, racism, unequal access to health care, and increased costs that are likely to be associated with genetic tests and procedures. How this will change if universal health care is implemented remains to be seen. Genomic technologies will provide new opportunities and their translation into healthcare applications should not be held back because of this concern. Genetic data collection should be extended to as many diverse populations as possible. It is also critical to assess nongenetic factors, which vary substantially among populations and may interact in important ways with genetic risk factors. Analyses of these effects and interactions can be especially powerful in the context of large, long-term prospective studies. To make the best use of genomic data, physicians and the public should be educated about its benefits as well as limitations. Such measures may help to reduce disparities in health.

## *Pharmacotyping*

Pharmacotyping is individualized drug selection and dosage profiling by the physician based on clinical evaluation of the patient's genotyping and haplotyping data for genes involved in the pharmacokinetics and pharmacodynamics of drugs in the body (Vizirianakis 2007). Pharmacotyping could be a new dimension of pharmacogenetics/pharmacogenomics and its application in routine clinical practice in the post-genomic era could better depict drug selection and dosage. This means a transition from a drug-selection process mainly based on the physician's own experience, into a more, highly integrated, information-based and computer-aided pharmacotherapy-based decision, thus making drug delivery digitized, more efficient and safer. Advances in in silico modeling for predicting ADME

(absorption, distribution, metabolism, and excretion) could be incorporated into this system. Progress in nanomedicine with nano-based systems for targeted drug delivery and pharmacogenomics are moving the drug-prescription process toward pharmacotyping, but the utility of this approach needs to be demonstrated by cost-effectiveness analysis (Vizirianakis 2011). In order to achieve major benefits for all patients worldwide, a multidisciplinary technological infrastructure should be organized in the healthcare system to address issues affecting regulatory environment, clinical pharmacology guidelines, education, bioethics and genomics data dissemination.

## Comparative-Effectiveness Research and Personalized Medicine

The American Reinvestment and Recovery Act of 2009 gave comparative-effectiveness research (CER) a large boost in funding over the following 2 years. Despite a consensus that better information about the relative effectiveness of different medical interventions is needed to improve the quality and value of care, some view CER with skepticism. However, by supporting comparative studies it might counteract the criticism that there is a paucity of studies comparing personalized with conventional care, and may help in promoting further acceptance of personalized medicine. Although CER's methods are not entirely new, the federal initiative will support research that is both more comprehensive – encompassing many more treatments and conditions, as well as more complete outcome measures – and more relevant to real-world clinical decisions than traditional clinical research (Garber and Tunis 2009). For example, large observational databases and pooled trial results can be used to learn more about the subgroups of patients who benefit from therapy. CER is not a panacea, but it is a key to individualized care and innovation, not a threat. An initiative to advance our knowledge about the effectiveness of clinical strategies can hasten the day when personalized medicine transforms health care.

## Medicine in the Year 2025

Medicine is evolving rapidly in the postgenomic era and some of the general advances anticipated by the year 2025 are:

- Pathomechanism of most of the currently known major diseases will be understood at the molecular level.
- Genomic, proteomics, metabolic data from various research and commercial sources will be integrated in clinical medicine.

- Marked increase in the number of validated biomarkers and their use for monitoring therapy.
- Most of the ethical and policy issues about genetic testing will be resolved and it will be a routine for some population groups.
- Pharmacogenetics will be applied to identify those at risk of adverse drug events from certain drugs.
- Improvements in targeted drug discovery and increase in pharmacogenomics-based clinical trials.
- Marked improvement in drug delivery technologies, particularly by use of nanotechnologies.
- Increased approval of cell and gene therapies.
- Nanomedicine will be established with impact on diagnostics, pharmaceuticals and personalized medicine.
- Preventive medicine will be well recognized with acceptance of presymptomatic diagnosis and pre-emptive treatments.
- Automation, robotics and informatics will be integrated into clinical medicine. A new initiative 'IT Future of Medicine' aims to integrate molecular data (especially genomic information) with anatomical, physiological, environmental, and lifestyle data in a predictive model approach – the 'virtual patient' – to enable the physician to design the optimal treatment for an individual patient (Regierer et al. 2013).
- Increasing implementation of electronic health records (EHRs).
- Most of the advances resulting from use of new technologies in medicine will occur in the areas of cancer, neurological disorders, and viral infections.

Pharmacogenomics is already used in clinical trials and will become the standard. Companies that do not use pharmacogenomic testing in drug development will lose out to the ones that do so. Personalized medicine should be widely available by the year 2025. Although some of the pharmacogenomic-based new drugs being discovered now may not have completed the development by this time, use of some of the older drugs is being individualized and several components of personalized medicine are being put into place now. Molecular and diagnostic tests have a shorter time to approval than drugs and some are already in the market. Low throughput genotyping for some disease biomarkers is already in use. Integration of diagnostics and therapeutics is also taking place and it is anticipated that personalized medicine will develop parallel with the introduction of pharmacogenomic-based medicines.

Genotyping will be for twenty-first century medicine what the x-rays were for twenty-first century clinical practice. Genetic testing will eventually improve predictions about what diseases we are predisposed to, the timing of their onset, their extent and eventual severity as well as which treatments or medications are likely to be efficacious or deadly. Genotyping, however, does not necessarily correlate with response to medications and other factors such as environmental have to be taken into consideration in personalizing treatment. Finally, all diseases do not require personalized treatment.

## Concluding Remarks About the Future of Personalized Medicine

In the year 1998, when the first monograph with the title ("Personalized Medicine" was published, there was little interest in this topic (Jain 1998). Currently, there is a tremendous interest in this topic. Suddenly many experts have appeared on this topic. Some of them have backgrounds in pharmacogenetics and pharmacogenomics, but had not made any efforts to integrate other emerging technologies into personalized medicine. Others accept that personalized medicine will come but try to put the date off into the distant future.

A report published by the Royal Society of UK in 2005 identified important areas of application and the problems facing development of personalized medicine, and concluded "its true potential may not become apparent for 15–20 years, during which time a great deal more information may become available about the practicalities of applying information derived from complex multifactorial systems in the clinic" (Anonymous 2005). This conclusion was disputed even though the Royal Society claims to have consulted a broad spectrum of persons and organizations involved in personalized medicine, because they ignored the most important players, the biopharmaceutical industry (Jain 2006). The Royal Society's view of personalized medicine seems to be restricted to pharmacogenetics/pharmacogenomics and ignores several other technologies such as pharmacoproteomics and metabolomics. If one reviews the progress in molecular diagnostics during the past decade, current developments have surpassed the forecasts. Molecular diagnostics that are already in the market, or would become available in the next 5 years, will fulfil many of the needs of personalized medicine. The concept of personalized medicine is being accepted by the medical profession, regulatory authorities, health insurance organizations, and the biopharmaceutical industry.

Some skeptics of the results of HGP, HapMap and genome-wide association studies say that the personalization of medicine will not occur before a decade after completion of the HGP. Actually personalized medicine started before sequencing of the human genome was completed, but received a considerable impetus in its development from advances in genomic technologies. Some of these are stated briefly as:

- Sequencing is becoming cheap enough only recently to look for rare variants, and that many common variants do have roles in diseases.
- Numerous sites on the genome, most of them near genes, have been implicated in common diseases. Although many more remain to be discovered, work can proceed to develop diagnostics and look for therapeutic possibilities of some diseases.
- The only way to find rare genetic variations is to sequence a person's whole genome. That approach is now becoming feasible because the cost of sequencing is dropping and $1,000 genome is now feasible. The price may go down even further.

- HGP has provided a common scaffold of sequencing where every gene and control element can now be mapped to its correct site on the genome, enabling all the working parts of the system to be related to one another. This is accelerating further progress.
- The genome sequence has facilitated the development of many powerful new techniques for exploring its meaning, e.g. chip sequencing, which gives researchers access to chromatin, the complex protein machinery that both packages the DNA of the genome and controls access to it.
- Data from the HapMap has enabled population geneticists to reconstruct human population history since the dispersal from Africa some 50,000 years ago. They can pinpoint which genes bear the fingerprints of recent natural selection, which in turn reveals the particular challenges to which the populations on different continents have had to adapt.
- The completion of the HGP provides a road map for thorough interrogation of ene functions. In addition to identifying novel transcription factor targets, current studies may shift our attention to genome-wide characterization of histone modifications and DNA methylation. The importance of this type of study is further echoed by the Human Epigenome Project. This requires genome-wide technologies with high-throughput capability.
- Genomewide association studies, despite critics, have yielded important new biologic insights into some common diseases or polygenic traits that facilitate efforts to develop new and improved treatments and preventive measures on the basis of these. The rapid progress being made through meta-analyses suggests that many more common variants conferring a risk of disease will be identified in the next several years, leading to increasing stability of individual risk estimates. Once risk estimates are more stable, the usefulness of genetic screening will need to be considered for each disease, and recommendations about potential interventions will need to be made for persons whose predicted risk exceeds some threshold. Appropriate guidelines are urgently needed to help physicians advise patients who are considering this form of genetic testing as to how to interpret, and when to act on, the results as they become more stable.

We do not have to wait for 15–20 years to realize the potential of personalized medicine. Also to state that it will take that long for personalized medicine to become mainstream raises the question as to what is required to justify the use of the term “mainstream” in medicine. There are no definite criteria by which this term can be applied to personalized medicine. Not all the diseases will need personalized medicines or combination of diagnostics with therapeutics. Application of new technologies and medicines depends on the personal judgment and decision of the treating physician in each case. Personalized approaches will be available and are expected to be used where they are deemed appropriate. In conclusion, the progress in personalized medicine and related technologies justifies a more optimistic view. There will be significant activity relevant to personalized medicine in the clinical as well as biopharmaceutical sectors in the US by the year 2020. The interest in personalized medicine is worldwide although the implementation may be delayed due

to socio-economic factors in some developing Asian countries. Japan, with an advanced healthcare system and a prominent position of research activity in genomic medicine, has good prospects for introduction of personalized medicine. China, which is making considerable advances in new biotechnologies and applying them in genomics and sequencing, has the facilities for developing personalized medicine. The US will be the first country to adopt personalized medicine on a large scale as the US healthcare system has undergone reforms referred to as "Obamacare" or universal healthcare introduced in 2013.

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