Kewal K. Jain

Textbook of Personalized Medicine

Springer

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Dedicated to Barack Obama,
President of United States who introduced
the bill titled "Genomics and Personalized
Medicine Act of 2006" in the US
Senate in 2006

Preface

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 selected studies done during this period in a readable format for the 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 K.K. Jain MD

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Abbreviations

ACE	angiotensin-converting enzyme
ADME	Adsorption, Distribution, Metabolism, Excretion
ADR	adverse drug reaction
CE	capillary electrophoresis
CF	cystic fibrosis
CNV	copy number variation
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
EPOE	apolipoprotein E
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
IP	intellectual property
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
MTHFR	methylenetetrahydrofolate reductase
NCI	National Cancer Institute
NIGMS	National Institute of General Medical Sciences

NIH	National Institutes of Health (USA)
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
TDM	therapeutic drug monitoring
TNF	tumor necrosis factor
TPMT	Thiopurine methyltransferase
ZFP	zinc finger proteins

Chapter 1
Basics of Personalized Medicine

Most of the current drugs are approved and developed on the basis of their performance in a large population of people and each drug is prescribed to all patients with a certain diagnosis. However, medicine is now 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 show the average response of a study group. There is considerable individual variation; some patients show no response whereas others show a dramatic response. Although approximately 99.9% of our DNA sequence is identical, the 0.1% difference between any two individuals (except identical twins) is medically significant. Buried within this small percentage of difference lie the clues to hereditary susceptibility to virtually all diseases. At the DNA level, this 0.1% difference translates into 3 million sites of genomic variation. Studies of structural variations (SV) in the human genome, cited later in this chapter, indicate that differences between individuals are much higher than 0.1%.

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 genomics-based drug development. 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 and other individual characteristics. A broader term is integrated healthcare, which includes development of genomics-based personalized medicines, predisposition testing, preventive medicine, combination of diagnostics with therapeutics, and monitoring of therapy.

Definition of Personalized Medicine

There is no officially recognized definition of personalized medicine. The term "personalized medicine" was used as the title of a monograph in 1998 (Jain 1998a) and started to appear in MEDLINE in 1999, but most of the literature

K.K. Jain, *Textbook of Personalized Medicine*,
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Table 1.1 Selected terms relevant to the concept of personalized medicine

Customized drug therapy
Genomic medicine or genotype-based therapy
Individualized or individual-based therapy
Information-based medicine
Integrated healthcare
Omics-based medicine: pharmacogenomics/pharmacogenetics/pharmacoproteomics
Predictive medicine
Rational drug selection
Systems medicine
Tailored therapy
Translational medicine

relevant to personalized medicine is still indexed under pharmacogenomics and pharmacogenetics. Various terms that are used to describe the concept of personalized medicine are listed in Table 1.1. Personalized medicine, also referred to as individualized therapy, 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. 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 not an adequate synonym for personalized medicine as other factors are also taken into consideration. Besides genomics, proteomic technologies have facilitated the development of personalized medicines and 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 on the basis of 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 on the basis of 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 has 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.

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 on the basis of 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.

It is obvious that the progress made during the past few decades surpasses that made in the whole of 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,

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.
6,000–3,000 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.
4,000–500 BC	Ayurveda, the ancient medical system of India with a blend of transcendental meditation and herbs, provided the first concept of individualized healthcare.
3,000 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.
16–18th 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 on the basis of "like cures like" by Samuel Hahnemann in Germany. Homoeopathic prescribing is highly individualized to a person's "constitutional picture" rather than to specific diseases.
19th 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.
20th 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.
20th century 2nd 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 1998a).
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.
2006	US Senator Obama (now President) introduced "Personalized Medicine" Act.
2008	Genetic Information Nondiscrimination Act passed in the USA.

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 very complex and contains about 3-billion nucleotides. In 2001, the total number of genes in the human genome was estimated to be 25,000, which was much less than earlier larger estimates by the International Human Genome Sequencing Consortium in 2001. By 2005, the three members of the International Nucleotide Sequence Database Collaboration (INSDC) – the European Molecular Biology Laboratory (EMBL) Bank, GenBank, and the DNA Data Bank of Japan (DDBJ) – reached a milestone as these databases for DNA and RNA sequences reached 100 gigabases of information. The 100,000,000,000 bases of genetic code, collected since 1982, comprise over 55 million sequence entries from more than 200,000 different organisms. This information was placed in the public domain where it has been freely accessible to the scientific community. The nucleotide sequence data bases enable researchers to share completed genomes, the genetic makeup of entire ecosystems, and sequences associated with patents. Earlier manual data entry into the repository has been replaced by new automated technology, robotics, and bioinformatics. Combined with decreased cost, these have fostered faster data collection.

The gene count of 25,000 came under scrutiny after the publication of the mouse genome in 2002 revealed that many human genes lacked mouse counterparts and vice versa. The possibility that some genes were misidentified was considered. To distinguish such misidentified genes from true ones, a research team at Broad Institute (Cambridge, MA) developed a method that takes advantage of another hallmark of protein-coding genes, i.e., conservation by evolution. The genes were considered to be valid if and only if similar sequences could be found in other mammals such as mouse and dog. Application of this technique invalidated a total of approximately 5,000 DNA sequences that had been incorrectly added to the lists of protein-coding genes, reducing the current gene estimate to approximately 20,500 (Clamp et al. 2007). This study suggests that nonconserved open reading frames should be added to the human gene catalog only if there is clear evidence of an encoded protein. It also provides a principled method for evaluating future proposed additions to the human gene catalog.

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 (bp). An average chromosome contains 2,000–5,000 genes within 130 million bp 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 bp. Nucleosomes are further compacted to solenoids which are packed into loops and each of these contains about 100,000 bps 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. Scientists have determined 99.3% of the euchromatic sequence of the X chromosome (Ross et al. 2005). They found 1,098 genes in the sequence, of which 99 encode proteins expressed in testis and in various tumor types. A disproportionately high number of Mendelian diseases are documented for the X chromosome. Of this number, 168 have been explained by mutations in 113 X-linked genes, which in many cases were characterized with the aid of the DNA sequence. Examples are defects in the gene responsible for Duchenne muscular dystrophy 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 ands 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 Chapter 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. Until recently, there was no reliable method to identify DNA structure from the sequence. 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.

Single Nucleotide Polymorphisms

Small stretches of DNA that differ in only one base are called SNPs and serve to distinguish one individual's genetic material from that of another. SNPs comprise some 80% of all known polymorphisms. Among the roughly 3-billion nucleotide bp (i.e., the "letters") that make up the genetic code, SNPs occur with a frequency of one per 500 bp so that there are approximately 6 million SNPs. Each gene contains approximately 5 coding SNPs, which likely effect the expression of the estimated 20,000–25,000 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. More than 9 million SNPs have been already generated in public databases using a large number of methods but only a small fraction of these are well characterized and validated (Kim and Misra 2007). Technologies for SNP genotyping are described in Chapter 2. Potential uses of SNP markers include prediction of efficacy and adverse effects of drugs.

Genotype and Haplotypes

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 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.

Genetic Variations in the Human Genome

Although many studies have been conducted to identify SNPs in humans, few studies have been conducted to identify alternative forms of natural genetic variation.

These include insertions and deletions as well as copy number variations (CNVs) in the genome.

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 bp
2. Expansions by only one bp (monomeric bp expansions)
3. Multi-bp 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-bp 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 SVs, 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. One study identified 255 loci across the human genome that contain genomic imbalances among unrelated individuals; half of these regions overlap with genes, and many coincide with segmental duplications or gaps in the human genome assembly (Iafrate et al. 2004). 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.

Variation in Copy Number in the Human Genome

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 previous assumptions 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 CNVs, 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 CNV rather than SNPs.

In a related study, the researchers propose that the thousands of differences found in comparisons of the human genome map assembled by Celera Genomics with that from the public Human Genome Project may be due to natural genetic variation rather than errors, as previously proposed (Khaja et al. 2006). The results of the study uncover substantial undescribed variation in humans, highlighting the need for comprehensive annotation strategies to fully interpret genome scanning and personalized sequencing projects. This discovery has implications for personalized genome sequencing, which will require reliable "reference" human genomes as a basis for comparison.

Structural Variants in the Human Genome

Structural variants (SVs) 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-bp 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 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 that are unique within the human genome to quickly and accurately determine genomic variations. Overall, more than 1,000 SVs were mapped and documented. This number of SVs among humans is much larger than initially hypothesized; many of the SVs potentially affect gene function. The breakpoint junction sequences of more than 200 SVs were determined with a novel pooling strategy and computational analysis.

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 two and five 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 possibly more flexible, than previously considered.

Mapping and Sequencing of Structural Variants 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 were 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 are more than about 6,000 bp 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 bp, 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 and 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 sequence the genomes of people from around the world.

In order to understand SV, 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 SV 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.

1,000 Genomes Project

The 1,000 Genomes Project, which started in 2008, is an international research consortium that is creating a new map of the human genome that will provide a view of biomedically relevant DNA variations at a resolution unmatched by current resources. Organizations committed major support to the project are: the Beijing Genomics Institute (Shenzhen, China), the Wellcome Trust Sanger Institute (Hinxton, Cambridge, UK), and the National Human Genome Research Institute (NHGRI) part of the NIH. The NHGRI-supported work is being done by the institute's Large-Scale Sequencing Network, which includes the Human Genome Sequencing Center at Baylor College of Medicine (Houston, TX), the Broad Institute of MIT and Harvard (Cambridge, MA), and the Washington University Genome Sequencing Center at Washington University School of Medicine (St. Louis, MO). In 2008, three companies that have pioneered development of new sequencing technologies joined the 1,000 Genomes Project: Life Technologies, 454 Life Sciences (a Roche company), and Illumina Inc.

The 1,000 Genomes Project builds upon the International HapMap Project, which produced a comprehensive catalog of human genetic variation – variation that is organized into neighborhoods called haplotypes. The HapMap catalog laid the foundation for the explosion of genome-wide association studies that identified more than 130 genetic variants linked to a wide range of common diseases, including type 2 diabetes, coronary artery disease, prostate and breast cancers, rheumatoid arthritis, inflammatory bowel disease, and a number of mental illnesses.

The HapMap catalog, however, only identifies genetic variants that are present at a frequency of 5% or greater. The catalog produced by the 1,000 Genomes Project will map many more details of the human genome and how it varies among individuals, identifying genetic variants that are present at a frequency of 1% across most of the genome and down to 0.5% or lower within genes. The 1,000 Genomes Project's high-resolution catalog will serve to accelerate many future studies of people with specific illnesses.

Human Variome Project

The Australian-led Human Variome Project (HVP) was established in 2006 to fulfill the need to catalogue information on variations or changes across the human genome and to make it accessible clinically. With the variation information available for only 3,000 of the more than 20,000 genes in the human genome, researchers were limited in understanding the role of genetic variation in human disease and to catalogue it completely and accurately. HVP has made progress with pilot projects, a new scheme for funding part of the effort, and planning committees aimed at creating information pipelines (Cotton et al. 2008). HVP participants are working to encourage the development and adoption of standards, define and reach consensus on ethical guidelines, develop automated data submission systems, support curation, and promote participation in developing countries.

Ultimately, the investigators hope to be able to develop systems whereby diagnostic laboratory DNA information is fed into the HVP to provide a much more comprehensive database. That requires methods for capturing both legacy data – disease-related mutations that have been published or are recorded in lab books – and new data from the literature and diagnostic laboratories. For example, the International Society for Gastrointestinal Hereditary Tumours (InSIGHT) started a project in 2007 to create a database of mutations associated with colon cancer. That project involves creating a pipeline for collecting new and old data and compiling it on the Leiden Open Variation Database. An InSIGHT pilot project is also aimed at compiling worldwide information on mutations in four genes of interest and their relationship to colon cancer.

On a broader scale, those spearheading the HVP are currently developing strategies and resources to help researchers set up variome projects around the world. They are now developing a protocol which researchers need to follow to collect mutations in their individual countries. In addition, the team has also come up with a new scheme to help pay for such massive collection and curation efforts. The "Adopt-a-Gene Program" is intended to give industry and patient support groups the opportunity to sponsor data collection on mutations in specific genes of interest. They see HNP as complementary to resequencing projects such as the 1,000 Genomes Project.

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 SNP 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, and 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.

- 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 the following:

- 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. These are discussed in more detail in Chapter 3.

Relationship of Various Technologies to Personalized Medicine

Relationship of various technologies to personalized medicine is shown in Fig. 1.1. Among various technologies nanobiotechnology will play an important role in the development of personalized medicine (Jain 2009m).

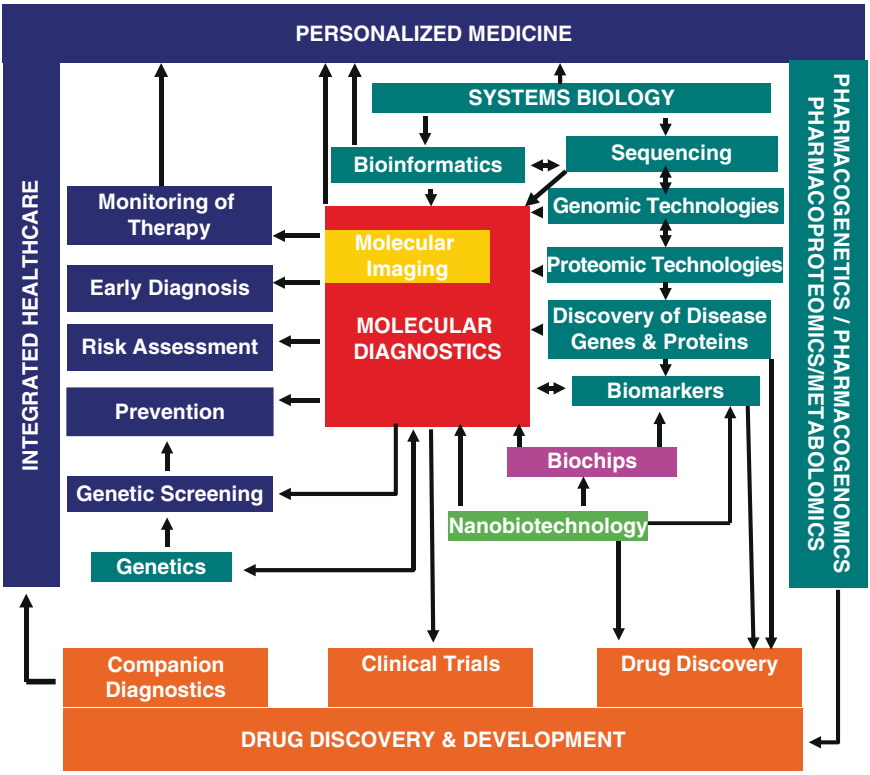

Fig. 1.1 Relation of personalized medicine to other technologies. © Jain PharmaBiotech

Conventional Medicine vs. 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.

The concept of personalized medicine is the best way to integrate new developments in biotechnology for the development of new drugs and diagnostics to improve healthcare. One of the most important contributions has been the sequencing of the human genome.

Genetic Basis of Personalized Medicine

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 in human 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 have identified approximately 100 loci for nearly 40 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 risk.

Mass Analysis of DNA from Whole Populations

Advances in technologies designed to obtain DNA sequence information are moving at a significant pace but current technologies can only analyze one genome at a time. Dr Sydney Brenner, winner of the Nobel Prize in Physiology or Medicine in 2002, has devised a new method for obtaining sequence information from thousands of genomes simultaneously, which will be developed by Population Genetics Technologies. It is expected to reduce significantly the cost of studying large populations of genomes. Such studies are important to the discovery of genetic variations

that affect common diseases and to the development of safer, more effective drugs. This new technology will enable users to discover extremely quickly much information about such gene variants from studies of whole populations. It can be used also for a broad range of complex biological problems requiring many parallel analyses. Examples are elucidating genetic changes in expressed genes in many samples of cancer, or understanding the different responses that people have to drug treatment, so as to better adapt medications to the needs of individual patients.

However the new method, if successful, will be a huge leap forward as it is expected to provide a significant cost advantage over other techniques which analyze one genome at a time, no matter how efficiently. This is because this method will allow the mixing of thousands of samples in one test tube and the simultaneous interrogation of all of them in one experiment, instead of in as many experiments as there are genomes in a population. Although pooling techniques that allow simultaneous analysis of multiple genomes have been used, these only provide population-wide characteristics, such as the frequency of gene variation, and not information specific to individual genomes. This technology will enable handling of much larger numbers of genomes than pooling does and will have the further advantage of protecting the identities of individuals involved in any population study by allocating them a code that may be kept confidential.

The technology might enable the discovery of mutations, rare in a clinical trial population, but responsible for serious deleterious side effects that are discovered only when the drug is very broadly prescribed. Patients that are potentially subject to such side effects could be screened if these mutations are determined.

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 have been collected on the classic Mendelian disorders and are 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/) help 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 on the basis of data from clinical trials.

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 is 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 the following:

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.

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.

Role of Systems Biology in Personalized Medicine

Scientists at the Institute for Systems Biology (Seattle, WA) have developed a concept of systems biology which 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. 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 (Voit et al. 2006).

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, preventive, and personalized approach to medicine (Weston and Hood 2004). 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) has set aside $7 million in the year 2009 to create two National Centers for Systems Biology in the USA. NIGMS 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 synthetic 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 enable 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. Relation of systems pharmacology to personalized medicine is shown in Fig. 1.2.

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

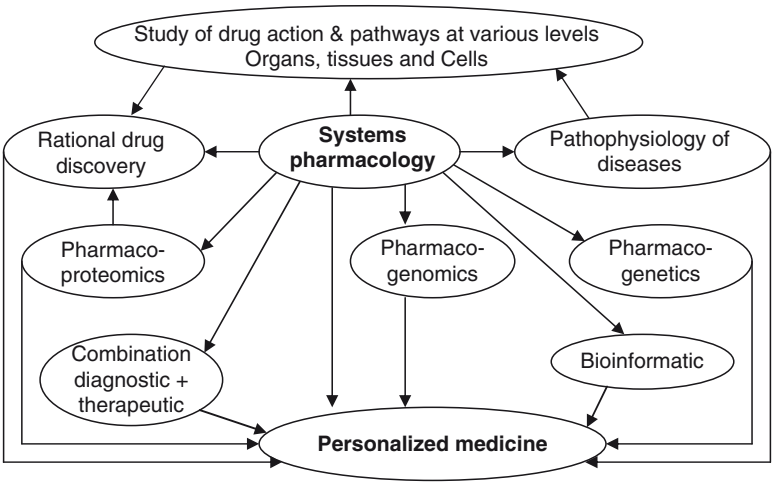

Fig. 1.2 Relation of systems pharmacology to personalized medicine. © Jain PharmaBiotech

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 (Auffray et al. 2009). 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 academy–industry partnerships that will target multiple components of networks and pathways perturbed in diseases. They 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.

A Personalized Approach to 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 for 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 and assessing human behaviors that influence the likelihood of exposure to environmental agents at a personal level. 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.

Reclassification of Diseases

Because all major diseases have a genetic component, knowledge of genetic basis helps in distinguishing between clinically similar diseases. Classifying diseases on the basis of 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 adapt therapies to the individual patients.

Several diseases can now be described in molecular terms. Some defects can give rise to several disorders, and diseases will be reclassified on molecular basis rather than according to symptoms and gross pathology. The implication of this is that the same drug can be used 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 markers 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 on the basis of 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 3 to 100 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.

Summary

This chapter defines personalized medicine and the basics. The scope is much broader than that indicated by the term "genomic medicine" and takes into consideration genetic, as well as epigenetic and environmental factors. Relationships to other technologies are shown as personalized medicine is the best way to integrate emerging technologies and translate them into clinical practice. The most important of these technologies of impact are molecular diagnostics. Systems biology approach to systems medicine is important for the development of personalized medicine.1

Chapter 2
Molecular Diagnostics as Basis of 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. 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 4.1)– 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 single nucleotide polymorphism (SNP) maps and haplotype markers, 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.

Molecular Diagnostic Technologies

Molecular diagnostic technologies have been reviewed in a detailed report on this topic (Jain 2009a). Molecular diagnostics are used for genetic testing and have the potential to be applied for genetic screening of large populations. They can also be

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used as adjuncts to clinical trials. A classification of molecular diagnostic technologies relevant to personalized medicine is shown in Table 2.1. Some of these technologies, which are used for mutation detection, overlap with technologies for detection of SNPs described later in this chapter. The two most important technologies relevant to personalized medicine are SNP genotyping and microarray /biochip.

DNA Sequencing

DNA sequencing was initially used only for research purposes but has now become a routine tool in molecular diagnostics. The technologies are described in a special report on this topic (Jain 2009b). An important characteristic of a diagnostic assay is the specificity of the nucleic acid sequence that is detected. Several research and clinical laboratories are now using DNA/RNA sequencing technology for the following applications that are relevant to personalized medicine:

- HIV resistance sequence analysis
- HCV genotyping
- Genetic diseases

Most new sequencing techniques simulate aspects of natural DNA synthesis to identify the bases on a DNA strand of interest either by "base extension" or "ligation." Both approaches depend on repeated cycles of chemical reactions. However, cost can be lowered and speed is increased by miniaturization to reduce the amount of chemicals used and to read millions of DNA sequences simultaneously. Several technologies are available for sequencing.

Biochips and Microarrays

DNA Biochip Technology for Developing Personalized Medicine

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 2009c). 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 less than 200 μm in diameter. It is comprised of DNA probes formatted on a microscale (biochips) plus the instruments needed to handle samples (automated robotics), read the reporter molecules (scanners), and analyze the data (bioinformatic tools). Selected applications of biochip technology relevant to personalized medicine are listed in Table 2.2.

Table 2.1 Examples of 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
Scorpions™ (DxS Ltd): closed-tube platform for the efficient homogeneous detection of PCR amplicons
Single-strand conformational polymorphism
Non-PCR methods
Arrayed primer extension
Enzyme mutation detection
Fluorescence 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 genotyping
DNA methylation studies
Gene expression based tests
DNA sequencing
Multiplex DNA sequencing
Sequencing in microfabricated high-density picoliter reactors
Whole genome sequencing
Cytogenetics
Comparative genomic hybridization (CGH)
Fluorescent in situ hybridization
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
Molecular imaging
Functional MRI with nanoparticle contrast
FDG-PET
Optical imaging
Point-of-care diagnostics

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
For storage of the patient's genomic information
Integration of diagnosis and therapeutics

Microarrays allow scientists to look at very subtle changes in many genes simultaneously. 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 severity of the disease and to identify the genes that can be targeted for therapy. This is how microarrays can potentially be used to develop personalized medical treatments. Figure 2.1 shows how the applications of biochips for pharmacogenetics and SNP genotyping form the basis for development of 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. 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. Microarray results can be comparable across multiple laboratories, especially when a common platform and set of procedures are used. Improving and standardizing microarray experiments will also enable early detection of diseases like cancer. This study may bring us one step closer to personalized medical treatment.

Numerous biochip technologies are available for clinical applications. The best known are the GeneChip (Affymetrix) and the AmpliChip CYP450 (Roche), which was cleared by the regulatory authorities for marketing in the US and the EU as an in vitro laboratory diagnostic test in 2004. The test is performed using DNA that is

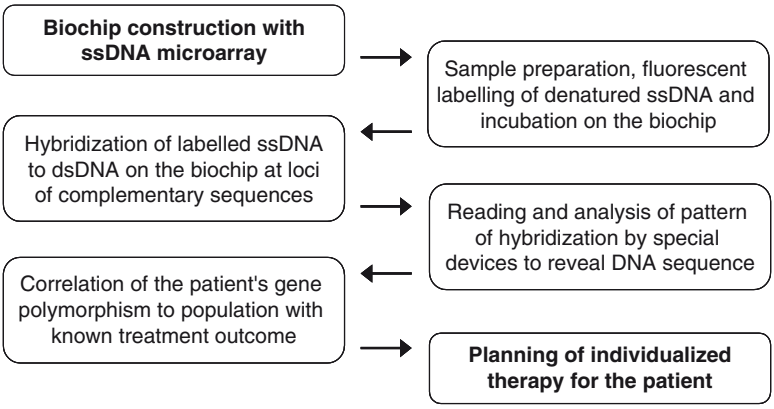

Fig. 2.1 Role of biochips/microarrays in personalized medicine © Jain PharmaBiotech

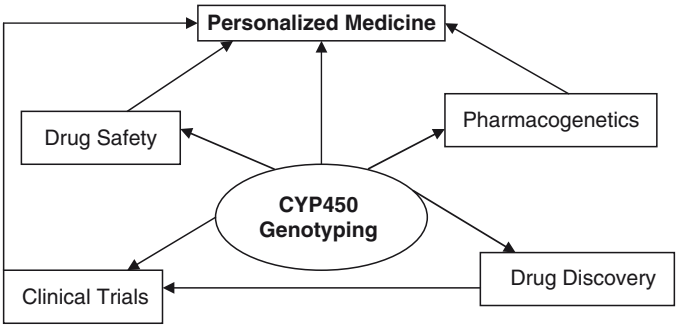

Fig. 2.2 Role of CYP450 genotyping in development of personalized medicine © Jain Pharma-Biotech

extracted from a patient's blood. DNA sequence is determined on the basis of the sequence of the probe molecule to which the DNA is most similar. AmpliChip CYP450 contains more than 15,000 different oligonucleotide probes to analyze both the sense and the antisense strands of an amplified target DNA sample (Jain 2005a). Virtually all known polymorphisms and alleles of CYP2D6, and the two most frequent for CYP2C19, can be detected simultaneously. AmpliChip CYP450 provides comprehensive coverage of gene variations, which play a role in the metabolism of approximately 25% of all prescription drugs. AmpliChip CYP450 test is intended to be an aid for physicians in individualizing treatment doses for patients on therapeutics metabolized through these genes. The role of CYP450 genotyping in development of personalized medicine is shown in Fig. 2.2.

Role of Protein Biochips in Personalized Medicine

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. 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.

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 microchips. Miniaturized and highly parallel immunoassays will greatly improve efficiency by increasing the amount of information acquired with single examination and reduce cost by decreasing reagent consumption.

ProteinChip (Vermillion, Inc.) has a role in proteomics comparable to that of GeneChip in genomics. It is based on SELDI (surface-enhanced laser desorption/ionization) process, which has four parts as applied to patient samples:

1. Patient sample of proteins is processed on the ProteinChip Array.
2. 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).
3. 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).
4. 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.

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 is yet to be developed. Sensitive and highly parallel technologies analogous to the nucleic acid biochip, for example, do not exist for protein analysis.

Protein chips will be particularly useful for clinical implementation of personalized medicine. 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.

Cytogenetics

The term "cytogenetics" has been classically used for studies of the cellular aspects of heredity. It has been used mainly to describe the chromosome structure and identify abnormalities related to disease. Besides clinical diagnostics, cytogenetics has been used for basic genomic research as well. It is better to include cytogenetics under the term "cytomics," which means that 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 nucleic acids, proteins, and metabolites (cellular genomics, proteomics, and metabolomics), as well as cell function parameters like intracellular pH, transmembrane potentials, or ion gradients. The broader scope of biology at cell level can be covered by terms such as cytogenomics, cytometabolomics, and cytoproteomics. Because of its important role in diagnosing disease at molecular level, cytogenetics is an important part of molecular diagnostics and can be referred to as molecular cytogenetics. Cytogenetic technologies are described in detail in a special report on this topic (Jain 2009n).

Molecular Cytogenetics as Basis for Personalized Medicine

Exciting advances in fluorescent in situ hybridization (FISH) and array-based techniques are changing the nature of cytogenetics, in both basic research and molecular diagnostics. Cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in carcinogenesis, and the 3D organization of the human genome. The high resolution that is achieved by these techniques, particularly by microarray technologies such as array comparative genomic hybridization, is blurring the traditional distinction between cytogenetics and molecular biology.

Classic cytogenetics has evolved from black and white to technicolor images of chromosomes as a result of advances in FISH techniques, and is now called molecular

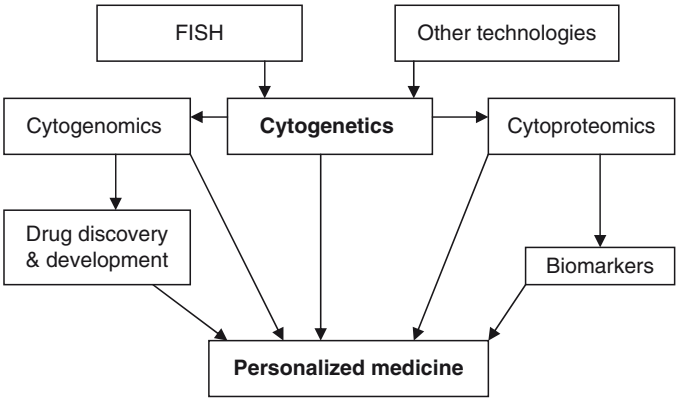

Fig. 2.3 Relation of cytogenetics to personalized medicine © Jain PharmaBiotech

cytogenetics. Improvements in the quality and diversity of probes suitable for FISH, coupled with advances in computerized image analysis, now permit the genome or tissue of interest to be analyzed in detail on a glass slide. It is evident that the growing list of options for cytogenetic analysis has improved the understanding of chromosomal changes in disease initiation, progression, and response to treatment.

The architecture of the human genome as revealed by the human genome sequencing project explains the recurrence of microdeletions and microduplications caused by a non-allelic homologous recombination involving segmental duplications created during the evolution of primates. The new data have greatly contributed to our understanding of human chromosomal diseases. Molecular cytogenetics will enable the further assessment of molecular basis of structural chromosome anomalies.

Cytogenetics is related to other technologies in the same way as genetics and hence to personalized medicine with the difference that everything is at cell level (Fig. 2.3).

Cytomics as a Basis for Personalized Medicine

In addition, differential molecular cell phenotypes between diseased and healthy cells provide molecular data patterns for (a) predictive medicine by cytomics or for (b) 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

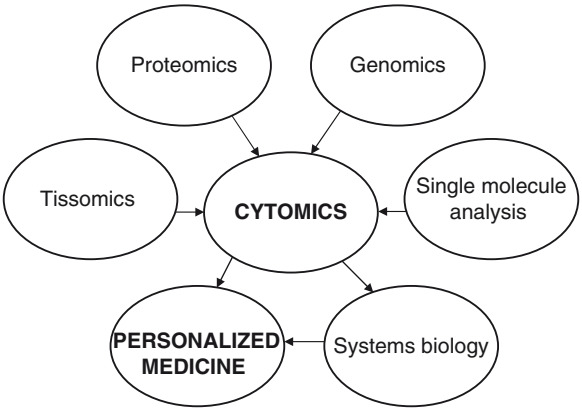

Fig. 2.4 Relation of cytomics to personalized medicine © Jain PharmaBiotech

cytome 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). Relation of cytomics to personalized medicine and other related technologies is shown in Fig. 2.4.

SNP Genotyping

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 2009a) but some are described briefly in the text following the table. Desirable characteristics of a genotyping technology are the following: (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, TaqMan assays and DNA microarrays are also frequently used genotyping methods.

Applications of SNPs Relevant to Personalized Medicine

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 opportunity to explore pathomechanism of human diseases and are unbiased by previous hypotheses or assumptions about the nature of genes that influence

Table 2.3 Technologies for SNP analysis

Digital Genetic Analysis
DNA chips and microarrays
DNA sequencing
Electrochemical DNA detection
Solution-borne ferrocene-modified DNAs
Redox-active intercalators
Surface-bound molecular beacon-like DNA
Fluorescence-detected 5′-exonuclease assays
Hybridization assays
Allele-specific oligomer hybridization
Array hybridization assays, e.g., MASDA (mutiplexed allele-specific diagnostic assay)
Hybridization with PNA probes
Invader assay
Mass spectrometry (MS)
Matrix Assisted Laser Desorption Ionization Time of Flight MS (MALDI-TOF MS)
Competitive Oligonucleotide Single Base Extension
Nanoparticle probes
Oligomer-specific ligation assays
PCR-based methods
PCR-CTPP (confronting two-pair primers)
Degenerate oligonucleotide primed (DOP)-PCR
TaqMan real-time PCR
Smart amplification process version 2
Peptide nucleic acid (PNA) probes
Primer extension
Pyrosequencing
Single base extension-tag array on glass slides (SBE-TAGS)
Single molecular fluorescence technology
Triplex Assay (Genetic Technologies, Inc.)
WAVE System's Temperature Modulated Heteroduplex Analysis method
Zinc finger proteins

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. 2.5.

SNPs 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.

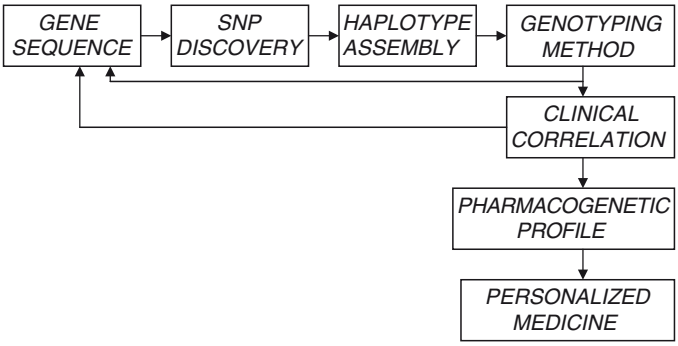

Fig. 2.5 Role of SNPs in personalized medicine. © Jain PharmaBiotech

- SNPs can be used as markers to segregate individuals with different levels of response to treatment (beneficial or adverse) in clinical settings.
- SNPs have a role in clinical trials as genotyping is important in design and interpretation of clinical studies.

Advantages of molecular genetic profiling in clinical studies are the following:

- 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.

Concluding Remarks on SNP Genotyping

Several methods are available for SNP genotyping. For ten or fewer SNPs and sample numbers in the thousands, the current gold standard is TaqMan real-time PCR (Life Technologies). MassARRAY system (SEQUENOM), is a mass spectrometry-based platform suitable for high throughput and up to 1,000 SNPs. Pyrosequencing (Biotage AB), 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 500K Array Set and Affymetrix GeneChip® Scanner 3000 MegAllele, based on Molecular Inversion Probe Technology, 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 100K chip with a 250K chip. Restriction fragment length polymorphism 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.

Pharmacogenetic capabilities have changed remarkably since the first SNP map from the SNP Consortium became freely available in 2001. It is now possible to use SNP-mapping technologies 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. Interindividual variability in drug response, ranging from no therapeutic benefit 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 soluble receptors (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 markers for susceptibility to familial rheumatoid arthritis treatment response to TNF inhibitors.

Haplotyping

An alternative approach to SNP genotyping is haplotyping. 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.

HapMap Project

Compared to the map of the human genome, which provides a route finder in genetics, a haplotype map will show the sites along the way. HapMap, a public resource created by the International HapMap Project (www.hapmap.org), is a catalog of genetic variants (SNPs) that are common in human populations. It will enable efficient and large scale studies in genetics and show common variants that cause disease. The HapMap project is the first major post-genomic initiative and is built on the experience gained from sequencing the human genome. The results will provide the physicians with basics of pharmacogenomics to enable them to give personalized treatments to their patients.

HapMap will accelerate the discovery of genes related to common diseases, such as asthma, cancer, diabetes, and heart disease. This information will aid researchers searching for the genetic factors that affect health, disease, and responses to drugs and the environment. HapMap is a shortcut to scanning through millions of SNPs. One need only to find blocks into which the genome is organized, each of which may contain several SNPs. SNPs in a haplotype block are inherited together and the pattern of SNPs in a haplotype block is unique for an individual. Currently this information is being used for the development of genetic panels to be used in pharmacogenomic and disease risk assessment studies. HapMap would be useful in the US where little is known of the geneology of the population. Some population groups, however, share haplotype patterns from their common ancestors. HapMap program would be superfluous in Iceland, where it is possible to isolate disease genes in the highly structured genealogy of Iceland for any disease with a prevalence of more than 0.2%.

The consortium's new goal is to build an improved version of the HapMap that is about five times denser than the original plan. This "Phase II" HapMap will take advantage of the rapid, high-throughput genotyping capacity of Perlegen Sciences to test another 4.6 million SNPs from publicly available databases, and add that information to the map. Perlegen received a $6.1 million award from the NIH's National Human Genome Research Institute (NHGRI) to add data on 2.25 million additional SNPs to HapMap. The new development, enabled by a partnership among multiple funding sources, will expand that effort and test virtually the entire known catalog of human variation on the HapMap samples. This will increase the density of SNP "signposts" across the genome from the current average of 1 every 3,000 bases to about 1 every 600 bases.

Successful genome-wide association studies are the most visible and exciting outcome of HapMap to date, with the large number of robust and highly replicated genetic associations with common diseases providing novel and unexpected

insights into the pathophysiology of disease (Manolio et al. 2008). The HapMap has also been invaluable in developing genotyping and analytic methods, and providing samples for validation of variation detection methods and standardization of laboratory processes. Application of these association findings is expected to produce new advances in the prevention and treatment of common diseases.

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 on the basis of each patient's genetic constitution.

Nanodiagnostics for 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 (10^{-9} m). Nanobiotechnology is the application of nanotechnology in life sciences and is the subject of a special report (Jain 2009d). Application of nanobiotechnology in molecular diagnostics is called nanodiagnostics and is described in a book on Nanomedicine (Jain 2008). 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 a single biological molecule from a single cell. This opens new opportunities and provides powerful tools in the fields such as genomics, proteomics, molecular diagnostics, and high throughput screening.

Various nanodiagnostics that have been developed will improve the sensitivity and extend the present limits of molecular diagnostics (Jain 2007). 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.

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, the one using cantilevers is the most promising. 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. 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.

Cantilevers for Personalized Medical Diagnostics

An innovative method based on cantilevers has been developed for the rapid and sensitive detection of disease- and treatment-relevant genes (Zhang et al. 2006). This method detects active genes directly by measuring their transcripts (messenger RNA (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 the targeted gene transcript to its matching counterpart on one of the cantilevers results in optically measurable mechanical bending. Differential gene expression of the gene 1–8U, a potential marker for cancer progression or viral infections, could 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. The new method complements current molecular diagnostic techniques such as the gene chip and real-time polymerase chain reaction (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. Cantilever arrays have potential as a tool to evaluate treatment response efficacy for personalized medical diagnostics.

Nanopore-Based Technology for Single Molecule Identification

As single molecules are driven through a nanopore by a voltage differential, the 3D charge profile of a molecule is measured by the field-effect transistors (FETs), enabling each molecule in the sample to be uniquely identified and precisely quantified. This method does not require fluorescent or other labels, thermal cycling, or optics. This technology offers the prospect to eventually correlate DNA and its expressed proteins with specific disease states using an inexpensive, disposable, and portable device. For example, the device has the potential to enable development of exquisitely targeted treatments using sequencing data both from a patient and from the disease-causing pathogen. Compared to other nanopore-based technologies for measuring molecules using electronic signals, the Eagle approach achieves a 1,000-fold higher sensitivity as a result of the FETs embedded in the nanopores. This technology could potentially be the first to enable the identification and measurement of both DNA and proteins in a single sample at the same time. The technology could have significant implications for advancing personalized medicine on the basis of its potential for faster, more efficient, and less expensive protein and nucleic acid identification.

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 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. Proteomic technologies are described in detail in a special report on this topic (Jain 2009e). Proteomics-based assays are considered to be a distinct group within molecular diagnostics and should not be confused with immunoassays although some proteomic technologies are antibody-based.

Technologies with the greatest potential are 2D PAGE, antibody-based screening, protein-binding assays, and protein biochips. 2D PAGE is combined with mass spectroscopy-based sequencing techniques, which identify 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. Role of proteomics in the discovery of biomarkers will be described in Chapter 3.

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 who 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 posttranslational 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.

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.

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
RNA amplification
Monitoring in vivo gene expression
Magnetic resonance imaging

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 for Gene Expression Studies

DNA microarrays have become the main technological workhorse for gene expression studies. To date, detection platforms for most microarrays have relied on short (25 base) oligonucleotides synthesized in situ, or longer, highly variable length DNAs from PCR amplification of cDNA libraries. Long (50–80 base) oligonucleotide arrays are now available and might eventually eliminate the use of cDNA arrays. 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 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 file 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 development of personalized medicine on the basis of 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, it is possible that expression of many genes can be visualized simultaneously inside single cells with high spatial and temporal resolution. Use of the nucleus as the substrate for parallel gene analysis can provide a platform for the fusion of genomics and cell biology and it is termed “cellular genomics.” This technique takes a snapshot of genes that are switched on in a single cell. Used on a breast biopsy or suspect skin mole, it could pick out the first one or two cells that have harmful genes and become malignant.

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 20,000–25,000 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. This allows the identification of novel splice variants that cannot be detected using oligonucleotide microarray technology. Comparisons of alternative RNA splicing repertoires will not only provide such expression markers but will also aid candidate gene selection for SNP analyses, defining the location of the relevant SNPs within the genes. An increased understanding of the mechanism of alternative splicing and the further characterizations of splice variants will have a significant impact on pharmacogenomics and development of personalized medicine. 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 SNPs that are relevant to pharmacogenomics.

Molecular Imaging and Personalized Medicine

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 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 disease 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.

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 to visualize gene expression. 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.

Glycomics-Based Diagnostics

Glycomics is the study of glycans, which are information-rich molecules, composed of complex carbohydrates (sugars or polysaccharides) that are often attached to proteins, lipids, and cells and it focuses on inflammatory therapies. Interactions between carbohydrates and proteins mediate intracellular traffic, cell adhesion, cell recognition, and immune system function. Glycans are downstream in the biological information flow and are therefore closer to the actual state of affairs. They can generate information, which is more relevant to the pharmacological aspects of drug behavior than either DNA or proteins by themselves.

Glycominds, Ltd.'s personalized medicine approach to inflammatory disorders is on the basis of glycan molecules. This approach has significant advantage over SNPs and other DNA-based pharmacogenomics assays because the patient's inflammation level is correlated to his history of infection and physiological state, not just to his DNA. Autoimmune research based on protein-glycan interactions generates superior analysis. Using GlycoChip® arrays, Glycominds measures binding

at the antibody level, including sub-types (i.e. IgG, IgM, and IgA), T-cell glycan adhesion, and glyco-related serum proteins. By combining its proprietary knowledge of protein-glycan interactions with its superior approach to inflammation biomarker research, Glycominds' strategy is to discover exceptional biomarkers that will serve as personalized medicine tests. These novel biomarkers open up an unexplored angle for drug pharmacodynamics. An example of the application of this technology is Glycominds' gMS™ assay for multiple sclerosis (MS) that will enable to stage the predicted disease activity and identify the most appropriate treatment strategy in patients presenting with a first demyelinating event. Patients who may benefit from disease modifying therapy could commence it earlier and more aggressively if needed. Conversely, patients who are not at immediate risk and might not benefit from therapy could transiently avoid the effects of inconvenient and costly treatments. Glycominds is sponsoring two studies, PRACTIMS and DECISION, to validate its MS marker.

Combination of Diagnostics and Therapeutics

Combination of diagnosis with therapeutics, wrongly referred to as "theranostic" is an important component of personalized medicine. A more appropriate term is "pharmacodiagnostic." The diagnostics is linked to the therapeutic substance to select patients who would be suitable for treatment by a particular drug. The drug and the diagnostic test are marketed together. There are several such combinations in the market particularly for the treatment of cancer.

Point-of-Care Diagnosis

Point of care or near patient testing means that diagnosis is performed in the doctor's office or at the bed side in case of hospitalized patients or in the field for several other indications including screening of populations for genetic disorders and cancer. POC involves analytical patient testing activities provided within the healthcare system, but performed outside the physical facilities of the clinical laboratories. It 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. The patients may even conduct the tests themselves at home. After the laboratory and the emergency room, the most important application of molecular diagnostics is estimated to be at the point-of-care. 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 approach that of high-volume analyzers used in clinical laboratories. POC diagnosis is important for the development of personalized medicine and various applications are listed in Table 2.6.

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

Table 2.6 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

test associated with POC testing. This is particularly true in the coronary care units of hospital emergency departments, where new cardiac marker 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, 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.

Point-of-care diagnosis is well known with simple biochemical tests such as blood glucose monitoring. Role of biochips for this purpose is still in development. Protein chips, particularly microfluidic immunoassays, appear to be likely to get to point-of-care first as several technical problems associated with use of nucleic acid chips 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 or near the patient. Hand-held diagnostic devices, biochips and electrochemical devices for the detection of DNA are particularly suited for point-of-care diagnostics. Nanotechnology would be another means of integrating diagnostics with therapeutics. Nanotechnology-based diagnostics provides the means to monitor drugs administered by nanoparticle carriers. Nanodiagnostic sensors might be incorporated in nanorobotic devices in the future for navigating the body to detect and destroy viruses or cancer cells.

Point-of-Care Diagnosis of Infections

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 able to 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. Infectio Diagnostic, Inc. is developing PCR-based tests for the under-1-h detection and identification of infectious agents, thus revolutionizing the decision-making process of health care professionals.

Detection, identification, and characterization of pathogens are 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.

Advantages vs. 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 at 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. 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 1,500 genetic tests are available including those that indicate susceptibility to cancer, neurological disorders, and heart disease.

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 focus 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.

Personal Genetic Service

A large number of companies offer tests 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. A selection of companies offers genetic screening tests directly to consumer, usually via Internet. This list does not include companies offering genetic testing only for paternity, athletic ability, etc. At least three companies – 23andMe, DeCode Genetics, and Navigenics/Affymetrix – 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. The best organized program is that of Navigenics/Affymetrix, which also provides genetic counseling.

Role of Diagnostics in Integrated Healthcare

Concept of Integrated Healthcare

Advances in medical genetics, molecular diagnostics, and genome-based medicines will enable integrated healthcare systems incorporating genetic screening, prevention, diagnosis, therapy, and monitoring. Diagnosis and therapy would be central in such a system as shown in Fig. 2.6. A suitable term to describe such a system has not been coined as yet. The term "integrative medicine" is applied to indicate integration of complimentary medicine in traditional. The first example of the combination of diagnostics and therapeutics was in the management of AIDS. HIV genotyping tests were used to detect resistance to antiviral drugs and molecular diagnostics tests were conducted for viral quantification to monitor therapy. The

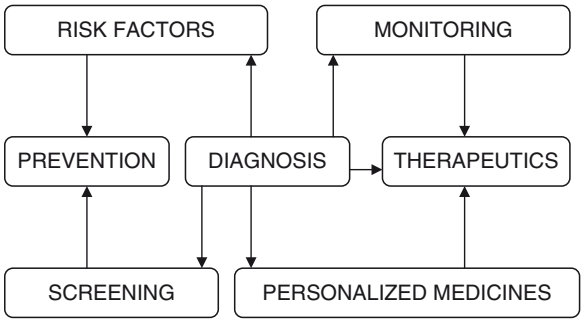

Fig. 2.6 A scheme of integrated healthcare and personalized medicine

initiative for development of such systems has come from the pharmaceutical industry as no academic or government organization has taken interest in this approach. Although the industry has a vested interest in the development of combined systems, there are advantages for the practicing physicians as well.

A combined system for diagnosis and therapeutics will have other components. The term diagnosis will broadly include screening for identification of risk factors, whereas therapeutics would also include monitoring of therapy. Prevention is added to this system because detection of predisposing factors can enable disease prevention by correction of risk factors or pre-emptive treatment. A key factor that will drive the integration of diagnostics and therapeutics is the availability of improved and more precise diagnostic methods, which are easy to perform and are not expensive. As discovery of disease genes progresses, the genes may form the link between diagnosis and gene-based medicines.

Components of Integrated Healthcare

Screening

It would be ideal to detect predisposition and risk factors before the development of a disease. The classical risk factors for major diseases are known but screening for genetic risk factors would be helpful in detecting specific risk factors for certain diseases. This would form the basis of preventive strategies. Search for disease targets is revealing a variety of molecular markers that can be used for molecular diagnosis, staging, and stratification of patient. Molecular diagnostics can be used for detection of disease predisposition. With increasing emphasis on preventive medicine, there will be an increasing emphasis on automated genotyping and individual risk profiling. Proactive identification of risk would enable prevention and management in a logical manner.

Disease Prediction

Predictive genetic testing is the use of a genetic test in an asymptomatic person to predict future risk of disease. These tests represent a new and growing class of medical tests, differing in fundamental ways from conventional medical diagnostic tests. The hope underlying such testing is that early identification of individuals at risk of a specific condition will lead to reduced morbidity and mortality through targeted screening, surveillance, and prevention.

Early Diagnosis

Early diagnosis of a disease before the symptoms appear is desirable but it is not possible for most of the diseases. Currently, early detection and treatment of disease

is on the basis of clinical chemistry methods combined with family history, lifestyle risk factors, and diagnostic imaging. Rapid advances, however, are being made in this direction.

Prevention

This could imply early detection and prevention of progression of a degenerative disease. Correction of risk factors may prevent either the development of a disease or its complications. Pre-emptive treatment may be on the basis of a correctable gene abnormality. In the conventional practice of neurology, it can be compared to repair of an intact asymptomatic intracranial aneurysm to prevent subarachnoid hemorrhage.

Therapy Based on Molecular Diagnosis

While the companion tests for therapeutic products themselves will be technically simple and most likely test for SNP variants, issues surrounding their development, regulatory approval, marketing, and reimbursement remain to be established. Therapy based on diagnosis is applicable to early, acute, or chronic stages of a disease. The patient may be treated by a medication determined to be safe and effective on the basis of molecular diagnostics. Not only would the cause of the illness be better defined by the molecular diagnosis, but also the most effective specific medication for the disease in a particular patient could be selected.

Monitoring of Therapy

Appropriate diagnostic tests can facilitate the frequent monitoring of the effects of therapy to verify the success by objective measurements and to detect the failure of therapy as early as possible so that appropriate changes in treatment can be instituted. Molecular diagnostic methods are an important part of monitoring of therapy.

Advantages and Limitations of Integrated Healthcare

Main advantages of the combined approach are as follows:

- A physician can provide comprehensive care for the patient without fragmentation of the components to several other physicians.
- Less wastage of ineffective costly therapies with financial savings and reduction of undesirable adverse effects for the patients. Expensive treatments may not be authorized without a definite diagnosis. Selection of drugs will be guided by unique genetic profile of the patient in order to optimize safety and efficacy.

- The patients themselves can conduct some of the tests under development.
- Genetic screening is linked to the treatment and if there is no treatment available for the genetic disorder, the patient may opt for foregoing the diagnostic test.

The interest of the biopharmaceutical industry is in packaging diagnostic and therapeutic materials to facilitate marketing. However, there are some limitations as follows:

- This approach cannot be universally applicable to all disorders.
- Not all the tests and treatments can be packaged together.
- The concepts of integration of various components in improving care of patients and reducing healthcare costs will need to be proven by further studies.

Nevertheless, the concept of integrating diagnosis and therapy, as well as monitoring, is a useful one for improving the general quality of healthcare in this age of super-specialization and fragmentation of care among numerous specialists who may not communicate well with one another.

Future of Molecular Diagnostics in Personalized Medicine

It is widely anticipated that the molecular diagnostic industry will continue to grow at double-digit pace to meet increasing demand for personalized medicine from 2008 to 2013. 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 at 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 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.

Summary

Molecular diagnostics includes some of the most important technologies for the development of personalized medicine. These are introduced briefly. Diagnosis at molecular level includes molecular imaging as well. These technologies will be

important for integration of diagnostics with therapeutics, which is an important component of personalized medicine. Apart from diagnosing disease, molecular diagnostics is used for determining the pathogenesis of disease, as well monitoring the effect of treatment.

Chapter 3
Role of Biomarkers in Personalized Medicine

Introduction

A biological marker (biomarker) is simply a molecule that indicates an alteration in physiology from the 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 marker. 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 special report on this topic (Jain 2009f). The expression of a distinct gene can enable its identification in a tissue with none of the surrounding cells expressing the specific marker. Relation of biomarkers to other technologies and healthcare is shown in Fig. 3.1.

Applications of biomarkers relevant to personalized medicine are:

- The biomarker would specifically and sensitively reflect a disease state and could be used for diagnosis, for predicting response to drug, and for disease monitoring during and following therapy.
- Biomarkers can be used as drug targets in drug development.
- Biomarkers might serve to integrate diagnostics and therapeutics.

Potential usefulness of biomarkers in development of personalized medicine is illustrated by the example of the discovery of 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 (Borovecki et al. 2005). The elevated mRNAs were 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.

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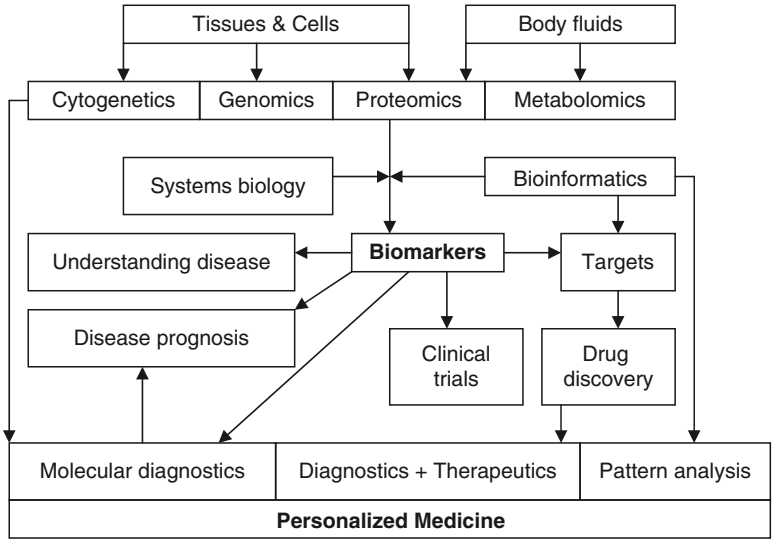

Fig. 3.1 Relation of biomarkers to other technologies and personalized medicine. ©Jain Pharma-Biotech

Technologies for Discovery of Biomarkers

Systems Biology Approach to Biomarker Identification

Ideally, a systematic approach to biomarker identification should involve multiple "-omic" technologies to investigate a disease process at all levels, including whole genome association studies to identify causative mutations or polymorphisms, as well as expression profiling, proteomics, and metabolomics to identify expression signatures and protein and small-molecule profiles that are either specific to the disease process or provide mechanistic insights into disease pathology. Uses of genomics, proteomics, and metabolomics in biomarker discovery are summarized in Table 3.1. Genomics is used to identify relevant disease genes, aberrant cellular signaling pathways and expression signatures correlated with disease. Proteomics is used to identify aberrant protein expression, post-translational modification, protein interactions, and protein profiles that are specific to a particular disorder. Finally, metabolomics is implemented to identify the presence of abnormal levels of small-molecule metabolites that are specific to and indicative of an underlying disease process.

Epigenomic Technologies

Epigenomics is one of the many 'omics' that have developed in the wake of the Human Genome Project. DNA methylation sites throughout the human genome

Table 3.1 Use of "-omic" technologies for discovery of biomarkers

Level of analysis	Tissue source	Technologies	Application
Genomics	Nucleated cells	Positional cloning	Mapping of disease loci
	Nucleated cells	SNP genotyping	Identification of disease gene
	Nucleated cells	Microsatellites	Mapping of disease loci
	Pathologically affected cells	Expression arrays	Identification of dysregulated genes
	Pathologically affected cells	Comparative genomic hybridization arrays	Detection of gene amplification and loss of heterozygosity
Proteomics	Affected tissues Body fluids: urine, blood, saliva	2D gel electrophoresis Liquid chromatography-mass spectrometry (MS) ICAT-MS	Identification of protein biomarkers
Metabonomics	Body fluids: urine, blood, saliva	Nuclear magnetic resonance (NMR) MS	Identification of small molecules
Glycomics	Body fluids: urine, blood, saliva	NMR Oligosaccharide arrays	Identification of carbohydrates Identification of glycoproteins

were mapped during Human Epigenome Project (HEP). The Human Genome Project provides the blueprint for life, but the epigenome tells us how this whole thing is executed, what determines when and where genes are switched on and off to produce a person. And knowing more about the human epigenome may provide clues to what goes wrong in cancer and other diseases. The latest information on this can be obtained at the HEP web site: http://www.epigenome.org/. As a prelude to the full-scale HEP, a pilot study of the methylation patterns within the Major Histocompatibility Complex (MHC) has been completed. This region of chromosome 6 is associated with more diseases than any other region in the human genome. Methylation variable positions (MVPs) were identified in the vicinity of the promoter and other relevant regions of approximately 150 loci within the MHC in tissues from a range of individuals. This provides an unprecedented insight into the complex relationship between genetics and epigenetics that underlies both normal cellular homeostasis and disease states, in particular autoimmune diseases. For the pilot project, an integrated genomics-based technology platform was developed. The pipeline involves the automated bisulphite treatment of DNA from minute tissue biopsies, gene-specific bisulphite PCR and large-scale sequencing of PCR amplicons. Analysis and quantification of methylation patterns is achieved by mass spectrometric and microarray assays.

Discovery of Methylation Biomarkers

Methylation is the only flexible genomic parameter that can change genome function under exogenous influence. Hence it constitutes the main and so far the missing link between genetics, disease, and the environment that is widely thought to play a decisive role in the etiology of virtually all human diseases. Methylation occurs naturally on cytosine bases at CpG sequences and is involved in controlling the correct expression of genes. Differentially methylated cytosines give rise to distinct patterns specific for tissue type and disease state. Such MVPs are common epigenetic markers. SNPs promise to significantly advance our ability to understand and diagnose human disease. DNA methylation is an important cellular mechanism modulating gene expression associated with aging, inflammation, and atherosclerotic processes. Global DNA hypermethylation is associated with inflammation and increased mortality in cardiovascular disease (Stenvinkel et al. 2007).

In the last few years, DNA methylation has become one of the most studied gene regulation mechanisms in carcinogenesis. Advances in the technologies that enable detection of DNA methylation in a variety of analytes have opened the possibility of developing methylation-based tests. A number of studies have provided evidence that specific methylation changes can alter the response to different therapeutic agents in cancer and, therefore, be useful biomarkers.

Proteomic Strategies for Biomarker Identification

Proteomics approach has been used to identify novel biomarkers. Although two-dimensional (2D) gel electrophoresis is used widely, ProteinChip has a greater potential for identification of biomarkers. Antibody arrays can be used for screening. Proteomic approaches for biomarker discovery have been used in many diseases. A 2D approach has been used for tumor marker identification in a number of cancers. Laser capture microdissection has been used in conjunction with ProteinChip to study protein expression profiles in cancer. The advantage of ProteinChip over 2D gel electrophoresis is that the chip platform used to identify the biomarker can also be used to develop a high-throughput assay.

Proteomics is a key technology for the discovery of biomarkers for pharmaceutical and diagnostic research. Although gene expression provides the level of proteins that is the key to the effect of the gene, it can be due to other factors in addition to the concentration of mRNA that codes for it. These factors include protein post-translational modifications, turnover, transport, and excretion. Therefore quantitative proteomics is essential for monitoring different pathways in blood samples of patients. Such biomarkers help in differential diagnosis as well as provide an understanding of pathomechanism of the disease and assessment of response to treatment. Non-invasive measurement (e.g., in serum) is the key feature of a biomarker that can be identified in diseased tissue. Multidimensional protein fractionation schemes are used to achieve appropriate sensitivity.

Proteomic Technologies for Detection of Biomarkers in Body Fluids

The first decision to be made in the search for a biomarker is whether to look in a body fluid or a tissue. Body fluids have the advantage of being more easily accessible and are more likely to be of clinical use because serum or urine can be obtained by non-invasive methods as a routine. Identification of rare proteins in blood is often hindered by highly abundant proteins, such as albumin and immunoglobulin, which obscure less plentiful molecules. A solution to this problem is an immunoaffinity column, Multiple Affinity Removal System (Agilent Technologies), which comprises antibodies to the six most abundant proteins found in human blood. By merely running a sample over the matrix, one can specifically remove all six proteins at once, unveiling lower-abundant species that may represent new biomarkers for disease diagnosis and therapy. The process removes about 85% of the total protein mass. The multiple affinity removal system works with blood, cerebrospinal fluid, and urine, all of which contain the same major proteins. Blood serum is the favored source for investigators interested in large-scale proteomics, because it has the most proteins. However, so far only about 500 of the 30,000 proteins in the serum have been identified. By removing albumin and the other five major proteins, scientists will be able to dig further into the proteome.

Not only has the number of proteins that can be detected in plasma expanded dramatically from hundreds to thousands, there is increased capability to detect structural variations of proteins. Recent studies also identified the presence of complex sets of small protein fragments in plasma. This set of protein fragments, the fragmentome or peptidome, is potentially a rich source of information about physiologic and disease processes. Advances in proteomics, therefore, offer great promise for the discovery of biomarkers that might serve as the basis for new clinical laboratory tests. There are many challenges, however, in the translation of newly discovered biomarkers into clinical laboratory tests. Only 10% of the proteins in human serum can be detected with currently available approaches, indicating the potential for further discovery of biomarkers. Protein variation is an untapped resource in the biomarker space, but only a selected few forms of proteomics applications are suitable for their analysis, and such variation could have a significant impact in disease diagnostics and therapeutic intervention (Kiernan 2008).

Biomarkers for 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.

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 markers. Use of proteomic technologies for detection of biomarkers will be a later section in this chapter. 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.

Biomarkers for Drug Development

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, 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.

Use of Biomarkers for Developing MAb Therapy in Oncology

The significance of pharmacogenomics in monoclonal antibody (MAb) therapeutics is highlighted by the association between polymorphisms in Fc receptors and clinical response to anti-CD20 MAb rituximab (Rituxan) or anti-ganglioside GD2 MAb 3F8, as well as the potential link between polymorphisms in HER2 and cardiac toxicity in patients treated with the anti-HER2 MAb trastuzumab (Herceptin). The dependence on gene copy number or expression levels of HER2 and epidermal growth factor receptor (EGFR) for therapeutic efficacy of trastuzumab and cetuximab (Erbitux), respectively, supports the importance of selecting suitable patient populations based on their pharmacogenetic profile. In addition, a better understanding of target mutation status and biological consequences will benefit MAb development and may guide

clinical development and use of this innovative therapeutics. The application of pharmacogenetics and pharmacogenomics in developing MAb therapeutics will be largely dependent on the discovery of novel surrogate biomarkers and identification of disease- and therapeutics-relevant polymorphisms. There are many opportunities as well as challenges in biomarker discovery and validation, and in implementing clinical pharmacogenetics and pharmacogenomics in oncology MAb development.

Biobanking, Biomarkers and Personalized Medicine

The Biobanking and Biomolecular Research Infrastructure (BBMRI, www.biobanks.eu), which started the preparatory phase in February 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 disease. Three types of biobanks have been considered as source of biomarkers in EU (Riegman et al. 2008).

1. Population banks. Their primary goal is to obtain biomarkers of susceptibility and population identity, and their operational substrate is germinal-line DNA from a huge number of healthy donors, representative of a concrete country/region or ethnic cohort.
2. Disease-oriented banks for epidemiology. Their activity is focused on biomarkers of exposure, using a huge number of samples, usually following a healthy exposed cohort/case-control design, and studying germinal-line DNA or serum markers and a great amount of specifically designed and collected data.
3. Disease-oriented general biobanks (i.e., tumor banks). Their goals correspond to biomarkers of disease through prospective and/or retrospective collections of tumor and no-tumor samples and their derivates (DNA/RNA/proteins), usually associated to clinical data and sometimes associated to clinical trials. Those data are usually not collected for a concrete research project, except in case of clinical trials, but from the healthcare clinical records. The amount of clinical data linked to the sample determine the availability and biological value of the sample.

Expression Signatures as Diagnostic/Prognostic Tools

Gene expression signatures as determined by microarrays can be used as biomarkers for diagnosis and monitoring of therapy. The best examples are in cancer. Ipsogen SA, has used gene expression signatures to refine molecular classes of breast cancer. Utilization of these signatures together with standard clinical parameters provides a unique combination discriminating patients responding to standard anthracycline chemotherapy. The test was validated in an independent cohort with patient samples from a multicenter clinical trial.

Althea Technologies Inc.'s proprietary eXpress Profiling™ multiplexed PCR technology, which enables high throughput gene expression analysis, is being combined with Natural Selection Inc.'s bioinformatics for discovery and application of gene expression signatures for a targeted disease or drug activity. This collaboration will provide advanced methods of data mining to extract biomarkers from the large gene expression data sets.

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 the preceding chapters. A few more examples are given here to show the value of biomarkers and their limitations in monitoring response to therapy.

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. It was determined by serum hepatic function tests and the 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) were used for monitoring the effect of lamivudine therapy for CHB (Maxwell and Flisiak 2005). Results showed that PINP/ITCP ratio is sensitive and specific in detecting responders to treatment.

Serial measurements of biomarkers might be beneficial to assess the adequacy of medication therapy for patients with advanced heart failure. Therapy guided by N-NT-proBNP, a biomarker of heart failure, might be helpful because NT-proBNP should be lowered by therapies that decrease endogenous BNP secretion. NT-proBNP and BNP were measured in a nonconsecutive patient cohort receiving clinically indicated intravenous nesiritide (Miller et al. 2005). In this study, many patients had decreased NT-proBNP and BNP values after therapy with nesiritide, but the majority of patients did not demonstrate biochemically significant decreases in analytes despite a clinical response. 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.

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 whose tumors overexpressed 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 the response is not wholly predictable. Reported response rates for HER2-positive cancers vary from less than 20% to more than 75%. HER2-positive cells that don't respond to Herceptin may have more active forms of the kinase Akt. And HER2 belongs to a receptor family that can be activated by 11 different soluble proteins and combinations thereof. Researchers are already betting that working out the biology behind the biomarker will lead to better treatments. Another anticancer antibody based on this understanding is already in clinical trials.

Similarly, the lung-cancer drug Iressa (gefitinib) could be rescued by a diagnostic based on a biomarker. Unfavorable clinical trial results dashed high hopes for big sales, but finding the patients most likely to benefit changed prospects. Various studies found that patients who responded to Iressa had mutations in the gene for EGFR.

Future Role of Biomarkers in Personalized Medicine

Personalized medicine is being recognized by the biopharmaceutical industry, regulatory authorities, healthcare providers, and the medical profession. It should be a part of the healthcare system by the year 2013 and will mature by 2015. Genetic testing will improve predictions of disease predisposition, onset, severity, and treatments or medications that are likely to be efficacious or harmful.

Summary

This chapter introduces biomarkers and technologies for their discovery. The important points on the role of biomarkers in the 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.Future Role of Biomarkers in Personalized Medicine

Chapter 4
Pharmacogenetics

Basics of Pharmacogenetics

Pharmacogenetics, a term recognized in pharmacology in the pre-genomic era, is the study of the 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 differences with small variability among patients 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 as an integration of gene profiling and pharmaceutical chemistry. From this initial definition, the scope has broadened so that it overlaps with pharmacogenomics.

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

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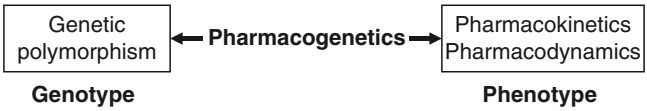

Fig. 4.1 Pharmacogenetics as a link between genotype and phenotype

Table 4.1 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

between pharmacogenomics and pharmacogenetics; both playan 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 4.1.

Role of Molecular Diagnostics in Pharmacogenetics

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

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.

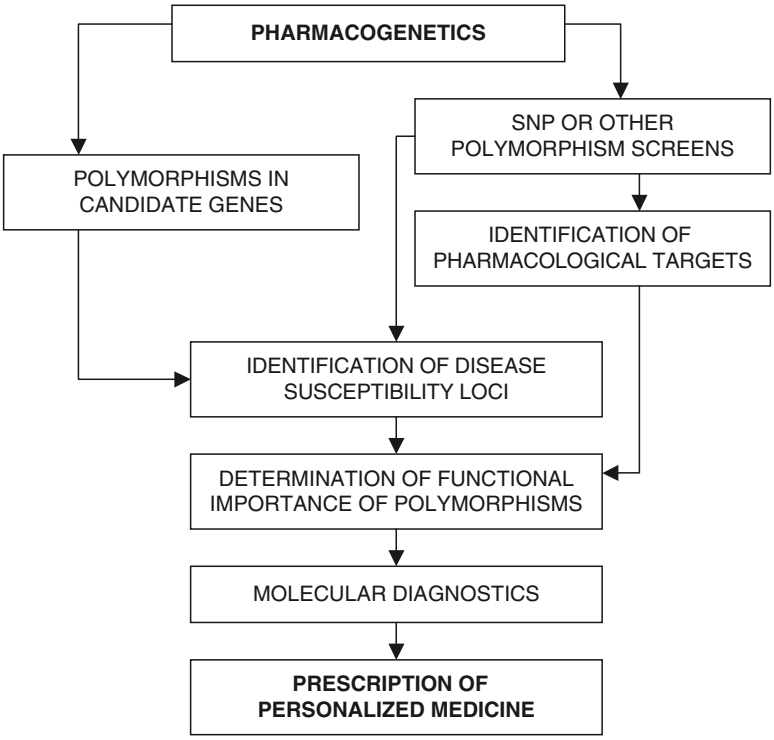

Fig. 4.2 Role of pharmacogenetic technologies in personalized medicine. © Jain PharmaBiotech

Role of Pharmacogenetics in Pharmaceutical Industry

Genes influence pharmacodynamics and pharmacokinetics. Pharmacogenetics has a threefold role in the pharmaceutical industry, which is relevant to the development of personalized medicines:

1. For the study of 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

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 such as 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 (ADR)
- 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 (G6PD) 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 4.2.

Table 4.2 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

Overall, in poor metabolizers (PM), 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 PMs under this condition will form only trace amounts of an active compound.

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 is likely to be 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 4.3.

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.

Table 4.3 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 4.4 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 4.5 Commonly prescribed medications, which are metabolized by CYP2D6

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

Frequency distribution of drugs metabolized by major CYP450 isoforms is shown in Table 4.4. Commonly prescribed medications, which are metabolized by CYP2D6, are shown in Table 4.5.

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 (PM) 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 (SSRIs)

Most reports of metabolic enzyme inhibition by 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 inter-subject variability in hepatocyte drug concentration is likely at the site of enzyme inhibition.

Positive and significant correlations between paroxetine and fluoxetine concentrations and CYP2D6 inhibition illustrate the role of plasma concentrations and dosage on magnitude of enzyme inhibition. The potential of paroxetine, a CYP2D6 substrateas 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) 38. 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% 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.

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 G6PD deficiency. G6PD, expressed in all of the body's tissues, controls the flow of carbon through the pentose phosphate pathway, produces nicotinamide adenine dinucleotide phosphate (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 and 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 subsequent 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. Among the 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 how 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 are 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 (SA) 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 SAs and they suffered from peripheral nerve damage, while fast acetylators were not affected. SAs are also at risk for sulfonamide-induced toxicity and can suffer from idiopathic lupus erythematosus while taking procainamide. The SA phenotype is an autosomal recessive trait. Studies have shown large variations of the SA 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 SAs. In East Asia, the further north the geographic origin of the population, the lower the frequency of the SA 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 clearly establish 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 (TDM) 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, which 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, only a 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, because only hepatoma cell lines have 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 that have been 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.

Another important gene family is the biogenic amine transporters, which regulate neurotransmitter levels in synaptic transmission. These transporters are the direct target receptors for numerous central nervous system (CNS) 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

Genetic variation in drug targets (e.g., receptors) can have a profound effect on drug efficacy. 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 4.6.

Table 4.6 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 with regression of coronary atherosclerosis
Arachidonate 5-lipoxygenase	Leukotriene inhibitors	Improvement in forced expiratory volume in patients with asthma
β_2-adrenergic receptor	β_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	Increase in bone mineral density
	Hormone-replacement	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 for chronic myeloid leukemia	A mutation in the Abl kinase domain of the Bcr-Abl gene may produce drug-resistance

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 in human cancers, inflammation, and metabolic diseases. Kinase SNP discovery programs are commercially available 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 Response of Disease to Drugs

Genetic Polymorphism of genes and gene products may influence the disease-modifying effects of drugs. Some examples are shown in Table 4.7. Such information is useful in identifying the responders to drugs and is discussed further in subsequent chapters.

Table 4.7 Effect of genetic polymorphisms on response of disease to drugs

Gene or gene product	Drug	Effect on response of disease 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

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 the 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. In most western populations, 93% are normal or efficient metabolizers (EM), 7% are PMs, 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 ratio (MR) greater than 12.6 and are homozygous for mutations. About 4% of the Caucasians are PMs of CYP2C19 when 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 a 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 midazolam is 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 no gender-related differences in pharmacogenetics but differences in pharmacokinetics may be related to drug-metabolizing enzymes. Men seem to have a higher activity relative to women for CYP P450 isoenzymes CYP1A2 and potentially CYP2E1, for the drug efflux transporter P-glycoprotein, and for some isoforms of glucuronosyltransferases and sulfotransferases. Women 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. For a few drugs, e.g., verapamil, beta-blockers and SSRIs, gender-related differences in pharmacokinetics have been shown to result in different pharmacological responses, but their clinical relevance remains unproven.

Role of Pharmacogenetics in Drug Safety

Variability in drug response among patients is multifactorial, including environmental, genetic factors besides the 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, 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 USA.
- Fatal ADRs are the fourth leading cause of death in the USA.
- 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 USA.
- 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 (McDowell et al. 2006).

ADRs in Children

The problems of ADRs in children are 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. ADRs in children caused by drug abuse are a major problem in the US. Children may be exposed to these drugs through ‘in utero’ 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.

In 2008, the NIH awarded grants to support 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 relevant studies.

Genetically Determined ADRs

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 ADR, 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 ADRs, and include many genes coding for drug-metabolizing enzymes. These genetic polymorphisms of drug metabolism produce the phenotypes of "PMs" or "UMs" 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 under the latter category. Mutant alleles at a single gene locus are the best studied individual risk factors for ADRs, including the genes for *N*-acetyltransferases, thiopurine methyltransferase, dihydropyrimidine dehydrogenase, and CYP450. However, pharmacogenetic factors rarely act alone; rather they produce a phenotype in concert with other variant genes such as those for receptors and environmental factors such as cigarette smoking. Examples of adverse reactions with a pharmacogenetic basis are shown in Table 4.8 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 significantly reduce the risk of drug toxicity.

Table 4.8 Examples of genetically determined adverse reactions to drugs

Drug	Adverse reaction	Underlying gene/mutation
6-mercaptopurine	Myelotoxicity, pancytopenia	Thiopurine
Azathioprine	Carcinogenicity	methyltransferase (TMPT)
β2-agonists	Increased airway reactivity	β2-receptor
Debrisoquin	Hypersensitivity	CYP2D6
Fluorouracil	Increased neurotoxicity	Dihydropyrimidine dehydrogenase
Fructose	Intolerance	Aldolase B
Inhalation anesthetics	MH	Ryanodine receptor
Irinotecan	Diarrhea Myelosuppression	Uridine diphosphate glucuronosyl transferase 1A1
Primaquine	Hypersensitivity: favism	G6PD
Proton pump inhibitors	Reduced efficacy in curing ulcers	CYP2C19
Sulfonal	Porphyria	Porphobilinogen deaminase
Suxamethonium	Hypersensitivity	Pseudocholinesterase
Typical antipsychotic drugs	Extrapyramidal effects, confusion Cardiotoxicity	Dopamine D3 receptor $5\text{-}HT_{2C}$ 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 et al. 2007):

- 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 (MH) in patients undergoing anesthesia. In other situations, it may help in the adjustment of dose of the drug such as in warfarin therapy.

ADRs of Chemotherapy

Neurotoxicity and myelotoxicity (manifested as neurtropenia) 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 has investigated pharmacokinetics as well. (Sissung et al. 2006). Patients carrying two reference alleles for the ABCB1 3435C>T polymorphism showed a reduced risk for developing neuropathy as compared to patients carrying at least one variant allele. Additionally, patients who were homozygous variant at the 2,677 and 3,435 loci had a significantly greater percent of decrease in absolute neutrophil count at nadir. Neither of the polymorphisms 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.

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. Research is ongoing 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 available in the USA 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, results from CARING (Clozapine and Agranulocytosis Relationships Investigated by Genetics) study led to the discovery of genetic biomarkers that enable identification of individuals at risk of developing clozapine-induced agranulocytosis. This may enable an approach to the prescribing of clozapine where a one-time genetic test may obviate the need for continuous blood monitoring for the majority of clozapine treated patients. These scientific 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 the development of a diagnostic test further. This gene is located in the human leukocyte antigen (HLA) complex, which has been previously reported to be associated with clozapine-induced agranulocytosis. Clinical Data Inc's PGxPredict:CLOZAPINE test makes it possible to provide patients with specific information about their probability of developing agranulocytosis in response to clozapine.

Role of Pharmacogenetics in Warfarin Therapy

Warfarin is the most commonly prescribed oral anticoagulant for the treatment and prevention of thromboembolic events. Approximately 2 million patients in the USA 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, which means that the safe dose range differs widely between individuals. Currently, complications of warfarin therapy account for 10.5% of the hospital admissions due to ADRs the second most common reason for patients to go to the emergency room. 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. In 2007, the FDA approved Verigene Warfarin Metabolism Nucleic Acid Test (Nanosphere Inc.), which detects variants of CYP2C9 and VKORC1 genes, responsible for sensitivity to warfarin. In the same year, the FDA cleared

Verigene® F5/F2/MTHFR nucleic acid test, which detects disease-associated gene mutations that can contribute to blood coagulation disorders and difficulties in 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).

Researchers at Uppsala University, together with colleagues at the Karolinska Institute in Stockholm (Sweden) and the Sanger Institute in the UK, conducted a genome-wide association study to find all the 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. 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 in the foreseeable future.

Role of Pharmacogenetics in Carbamazepine Therapy

Carbamazepine is responsible for severe ADRs such as Stevens-Johnson syndrome and toxic epidermal necrolysis 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 carbamazapine to warn patients of a genetic link to potentially serious side effects of carbamazepine and it plans to test 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. The clinical application of the results is somewhat limited as they are based on retrospective studies. Therefore, a series of preventive prospective studies are planned to assess the clinical applications of the risk genes and to determine if genetic screening can effectively reduce the incidence of ADR.

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 rare cases, myopathy occurs in association with statin therapy, especially when the statins are administered at higher doses and with certain other medications. A genomewide association study of patients on simvastatin therapy has identified SNP rs4363657 located within SLCO1B1 on chromosome 12, which 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 (The 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 of r developing this adverse reaction.

FDA Consortium for Genetic Biomarkers of Serious Adverse Events

In 2007, the FDA's decided to create a consortium that aims to observe how genetic biomarkers contribute to serious adverse events (SAEs) with members of the pharmaceutical industry and academia. It 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 is not in the best interests of the patients that the drug manufacturers simply launch these products without putting appropriate information on labels. SAE consortium (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 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 biomarker. SAEs, which include hepatotoxicity, rhabdomyolysis, 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 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 new group would be 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 besides 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.

Therapeutic Drug Monitoring, Phenotyping, and Genotyping

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 TDM has been used to eliminate variable pharmacokinetics as a source of nonresponsiveness as well as ADRs. 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 ADRs
- 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. Phenotyping enables rapid discovery of new and useful biomarkers, which will be useful for improving diagnosis and treatment of diseases as well as for developing better 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 molecule, cell and organism levels. New technologies and approaches will

therefore be required. Recent 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 co-administered 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 been applied successfully in some pharmacogenetics conditions such as MH, porphyries and G6PD 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 4.9.

Table 4.9 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 mani festations		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
G6PD 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

Phenomics

Phenomics is the study of genomics information to better understand the complex relationship between genotype and phenotype. This relationship is frequently non-linear 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.

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 transgenic 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 (DNAPrint Inc) will help identify an individual who is predisposed to develop cancer before the onset of illness so that lifestyles can be altered and/or preventative measures be 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, there are potential difficulties and problems in carrying them out. 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 can be clarified by metabolomic tudies.

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. 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 Chapter 2. Use of microarray technologies for toxicogenomics will be described later in this chapter.

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.

Gene Expression Studies

Gene expression is used widely to assess the response of cells to various substances. The following examples illustrate their 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 several thousand gene expression events.

Gene Profile Assays (Xenometrix). Gene profiling is the process whereby the status of gene expression in a given cell line is assessed at increasing concentrations of exposure to a test substance (such as a pharmaceutical). Xenometrix Gene Profile Assays (GPA) assesses gene expression through the use of cell-based assays and specific reporter constructs. These constructs report the activity of certain genes in a quantifiable process, determined at the conclusion of the exposure period. Through the assessment of the activity of key genes, information on the biological activity of the test compound can be gathered, and by including genes relevant to safety or efficacy concerns in the assay, the assay itself can be focused on these critical areas.

Molecular Imaging. In vivo gene expression can be monitored by molecular imaging. This has been applied to drug development at preclinical stage to study drug toxicity.

Genomics and the Prediction of Xenobiotic Toxicity

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.

Pharmacogenetics in Clinical Trials

Currently, the most significant polymorphisms causing genetic differences in phase I drug metabolism are known and therapeutic failures or ADRs 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 may 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. Benefit of application of this approach needs to be verified in prospective clinical trials using the parameters of reduction in ADRs, 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 SAEs 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 quantify 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.

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. These can be detected by AmpliChip CYP450 which was described in Chapter 2. 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 has 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 2008).

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 inter-patient variability or variations in expression in some of the polymorphic enzymes are of interest to 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.
- 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 SSRIs interact with CYP2D6 enzyme. The most notable example of this is fluoxetine. Through competition with CYP2D6 substrates, these drugs precipitate a drug-induced PM 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.

Examples of use of Pharmacogenetics in Clinical Pharmacology

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 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). Genotyping studies on patients with CDLE show a clear-cut difference in the outcome of treatment according to whether the patients are SAs rapid acetylators (RAs). Patients who respond to treatment with a complete or marked remission of the disease are usually RAs. Patients who do not respond at all to the drug are usually SAs. In addition, SAs seem to be more prone to toxic events. These findings strongly suggest that the genetic polymorphism of NAT2 is responsible for differences in the response to sulfasalazine in patients with CDLE. Therefore, candidates for sulfasalazine therapy should be genotyped to identify those patients who might benefit from the drug.

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 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 showed lack of efficacy.

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, the most common variant allele for low activity, an allele that encodes a protein with two changes in amino acid sequence, 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. The results of these studies 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.

Linking Pharmacogenetics with Pharmacovigilance

Genetic Susceptibility to ADRs

A non-invasive method that would be acceptable to members of the general population and also enable estimation of the risks that specific genetic factors confer on susceptibility to specific ADRs, involves use of buccal swabs to obtain cells for DNA extraction. A small pilot study of the method was conducted in the New Zealand Intensive Medicines Monitoring Program in 2004 to link prescription event monitoring (PEM) studies with pharmacogenetics. It was concluded that the use of buccal swabs is acceptable to patients and provides 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 ADRs using drug cohorts from PEM studies are possible, and there are several areas where population-based studies of genetic risk factors 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 prescribed guidelines, based on clinical studies, for drugs that are subject to substantial polymorphic metabolism
- Prescribing advice that 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 ADRs
- 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.

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 ADRs.

Summary

Pharmacogenetics, the study of influence of genetic factors on action of drugs, is the oldest and one of the important basics of personalized medicine. This chapter compares pharmacogenetics with pharmacogenomics and describes the role of molecular diagnostics in studying pharmacogenetics. Because genes influence the action and toxicity of drugs, pharmacogenetics plays an important role in drug development and drug safety. Enzymes relevant to drug metabolism are described

and the most important of these is cytochrome P450. Genotyping also predicts metabolic capacity but involves identification of defined genetic mutations that give rise to the specific drug metabolism phenotype. Clinical implications of pharmacogenetics including its use in clinical trials and medical practice have been discussed. There is a need for integrating pharmacogenetics in healthcare to develop personalized medicines that are safe for individuals.

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 the genes in an organism – their sequences, structure, regulation, interaction, and products. Currently, it is estimated that there are 20,000–25,000 genes in the human organism according to different estimates. 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. The 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 base for the development of personalized medicines.

Pharmacogenomics is 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. The 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 these complex multifactorial situations to obtain individual genotypic and gene expression information. These same tools are used to study the diversity of drug

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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

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, before prescribing, test patients for specific Single nucleotide polymorphism (SNPs) known to be associated with non-therapeutic drug effects 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 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 candidate genes potentially useful for drug discovery. Information obtained from a study of the function of genes, their interactions, their role in biological pathways, and 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.

Another important stage in drug discovery is lead selection that can be based equally upon markers of toxicity or markers of efficacy. A mRNA transcript profiling technology coupled with a database search, enables creation of 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. Such biomarkers can help triage hepatotoxicity and cardiotoxicity among other response profiles and reduce the cost of drug development.

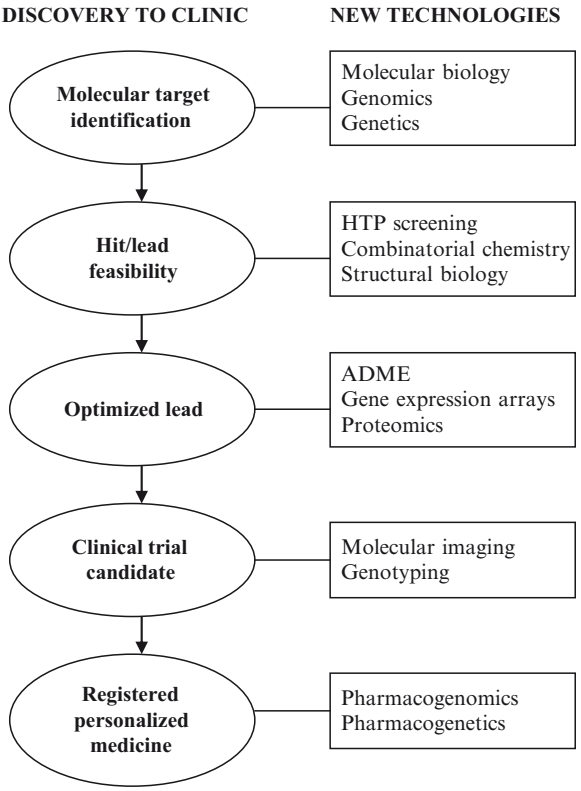

Fig. 5.1 Impact of new technologies at various stages of the drug discovery process. © Jain PharmaBiotech

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 2009 will undoubtedly play a role in the outcomes in 2013.

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 (AD) 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. A method of predicting the general therapeutic classes into which various psychoactive drugs fall is based on high-content statistical categorization of gene expression profiles induced by these drugs (Gunther et al. 2003). Using the classification tree and random forest supervised classification algorithms to analyze microarray data it is possible to derive general "efficacy profiles" of biomarker gene expression that correlate with antidepressant, antipsychotic, and opioid drug action on primary human neurons in vitro. These profiles have been used as predictive models to classify naive in vitro drug treatments with 83.3% (random forest) and 88.9% (classification tree) accuracy. Thus, 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.

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

The various roles of pharmacogenomics in clinical trials are listed in Table 5.2.

The knowledge of pharmacogenetics and pharmacogenomics is already improving the conduct of clinical trials based on genotyping stratification and development of personalized medicine. 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. Fig. 5.2 shows the various steps for the application of pharmacogenomics in clinical trials. Some examples of the use of pharmacogenomics in clinical studies are shown in Table 5.3.

Impact of Genetic Profiling on Clinical Studies

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

- It is a contribution to the molecular definition of the disease.
- It provides the correlation of drug response to the genetic background of the patient.

Table 5.2 Role of pharmacogenomics in clinical trials

Identification of variations in a 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

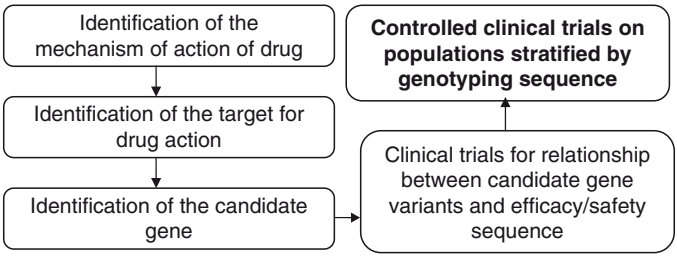

Fig. 5.2 Steps in the application of pharmacogenomics in clinical trials. © Jain PharmaBiotech

Table 5.3 Examples of pharmacogenomics-based clinical studies

Disease	Drug	Polymorphism	Results
Asthma	Zileutin	ALOX5 genotype	Reduced response among heterozygotes
AD	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

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- It predicts the 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.
- The 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 of phase II 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 phase II and analysis of data to identify responders, 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 the pharmacogenomics-based clinical trials are:

- Exclusion of certain subjects from the trials on the basis of genotype is interpreted as 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 the 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 who obtained treatment, by chance, that would have been recommended on the basis of genotype and the individuals who received inappropriate treatment. This approach could produce valuable data to support the value of pharmacogenomic testing.

Pharmacogenomic Aspects of Major Therapeutic Areas

Oncogenomics

Oncogenomics is the study of cancer genes. Cancer is a multifactorial disease involving interaction of environmental, hormonal, and dietary risks in addition to genetic predispositions. However, progression of a single cell from a normal to a neoplastic state always involves a series of genetic changes that alter either the regulation or the function of a variety of different genes. Such genes may play roles in a number of overlapping physiologic processes, including genome maintenance, cell cycle control, apoptosis, contact inhibition, invasion and metastasis, or angiogenesis. These cancer genes are often classified into two main categories, oncogenes and tumor suppressor genes. The distinction between these two categories is that tumor progression is promoted by overexpression or gain of function in oncogenes but by nonexpression or loss of function in tumor suppressor genes. Most highly penetrant cancer predispositions are thought to be caused by germline mutations in tumor suppressor genes but the same phenomenon can occur with germline mutations in oncogenes. For example, rare germline mutations in the ret proto-oncogene (RET) tyrosine kinase predispose to endocrine neoplasms.

Oncogenes

Oncogenes are genes associated with neoplastic proliferation following a mutation or perturbation in their expression. These genes, which form part of the signal transduction pathway, include growth hormones, receptors, G proteins, protein kinases, transcription factors, and cyclins.

The concept of an oncogene originated with the discovery of certain viral genetic elements that are responsible for the tumor-forming ability of retroviruses. The antecedent genes, known as proto-oncogenes, play an essential physiological role in normal cellular proliferation and differentiation. Although proto-oncogenes cannot form

neoplasms in their native state, they can induce cancer when they are captured and subverted by retroviruses (RNA viruses). Several proto-oncogenes have been described, including a number of them that are translocated (chromosomal translocations) in human cancers. In general, these genes appear to act on the biochemical pathways through which growth factors stimulate cellular proliferation. For example, overexpression or gain-of-function mutations in the proto-oncogene HER2/rieu, a member of the epidermal growth factor receptor family, constitutively activate a signaling pathway that promotes progression through the G1 phase of the cell cycle. At the cellular level, oncogenes act in an autosomal-dominant fashion; one abnormal copy of 1 allele of a proto-oncogene is sufficient to promote tumor progression

Tumor Suppressor Genes

Tumor suppressor genes or "anti-oncogenes" represent a new class of cellular genes that regulate cell growth by counteracting the action of proto-oncogenes. Their exact role has not yet been defined. At the cellular level, tumor suppressor genes function in an autosomal-recessive fashion. In a single cell, loss of function of both alleles of a tumor suppressor gene is usually required to promote tumor progression. Potential processes in which these genes might inhibit the development of cancer include cell proliferation, differentiation and senescence, cell-to-cell communications, and chromosomal stability.

The list of tumors associated with homozygous loss of specific chromosomal loci is growing rapidly. In addition, in vitro evidence supports the existence of tumor-suppressing genes (Table 5.4). To create these genes, fusion of a normal cell with a malignant cell produces a hybrid in which the carcinogenic phenotype is usually suppressed; the differentiation program of the normal parent cell may then be imposed upon this hybrid.

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 polymerase chain reaction (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 the molecular basis of cardiovascular disorders.

Table 5.4 Tumor suppressor genes, their chromosomal location, function, and associated tumors

Genes	Chromosomal locations	Functions	Associated tumors
APC	5q21	β-Catenin binding, communicates between cell surface proteins and microtubules	Familial adenomatosis polyposis coli
BRCA1	17q21–22	Tumor suppressor gene (unknown function)	Inherited susceptibility to breast and ovarian cancer
BRCA2	13q12–13	Tumor suppressor gene (unknown function)	Hereditary breast cancer
CDK4	12q13	Cyclin dependent kinase	Hereditary melanoma 2
p16 (*CDK2A*)	9p21	p16-Cyclin-dependent kinase inhibitor	Germline mutations cause hereditary melanoma
DCC	18q21	cell adhesion	Colorectal cancer
EXT1	8q24.1	Tumor suppressor gene (unknown function)	Langer Giedion syndrome
FHIT	3p24.3	Tumor suppressor gene altered by exposure to environmental agents	50% of gastrointestinal cancers
MSH2	2p16	Mismatch repair genes	Hereditary non-polyposis colorectal cancer
MLH1	3p21		
PMS2	7p22		
NF1	17q11.2	GTPase activating protein (GAP) for ras from neural crest derived cells	von Recklinghausen's neurofibromatosis
NF2	22q11.1	Integration of cytoskeleton with plasma membrane	Acoustic neuroma, bilateral meningiomas
P53	17p13	Transcription factor, regulates cell cycle, and apoptosis	Germline mutations cause LiFraumeni syndrome
PTC	9q22.3	Membrane protein involved in Hedgehog protein signal transduction	Basal cell carcinoma
RB1	13q14	Regulates transcription factors (E2F-DP1), regulates cell cycle	Retinoblastoma
RET	10q11	Receptor tyrosine kinase	Medullary thyroid cancer. Multiple endocrine neoplasia 2
TSC2	16p13	Tumor suppressor gene (unknown function)	Tuberosclerosis 2
VHL	3p25	Elongin (transcription elongation)	von Hippel-Lindau syndrome
WT1	11p13	Zinc finger transcription factor	Wilm's tumor, nephroblastoma

The number of genes expressed in the cardiovascular system is approximately 20,000 as the total number of genes is now considered to be ~25,000. Reported polymorphisms relevant to cardiovascular disease management are shown in Table 5.5. Genotyping for cardiovascular disorders and 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, β_1 and β_2 receptors, and endothelin in over 1,000 patients.

In genetic mapping of a large family with several members affected by a type of heart failure called dilated cardiomyopathy (DCM), additional mutations were found in a gene on chromosome 3 called SCN5A (Olson et al. 2005). SCN5A encodes the sodium ion channel in the heart, which helps regulate transport of positively charged sodium ions, and therefore the heart's electrical patterns. Among the individuals with an SCN5A mutation, 27% had early features of DCM, 38% had full-blown DCM and 43% had atrial fibrillation, a rhythm abnormality of the heart. These findings broaden the indications for genetic screening of SCN5A beyond isolated rhythm disorders. Since these variations hinder sodium transport, it is advisable to avoid using sodium 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, the 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.

Table 5.5 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 arrythmias 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

Neurogenomics

Neurogenomics covers neurological and psychiatric disorders even though these disorders belong to different clinical specialties. More than 50% of the genes in the human genome are expressed in the nervous system and an understanding of the function of these genes will contribute to our understanding of neurological disorders. The term neuropharmacogenomics refers to the genomic basis of drug action and stems from neurogenomics – the study of genes in the nervous system (Jain 2001a). Through the use of microarray/biochip technology, coupled with data bases of information about SNPs in potential candidate genes or risk factors for psychiatric disorders, it should be possible in the near future to stratify clinical populations genetically for inclusion in specific drug treatment trials. The ultimate goal of this research is to obtain homogeneous populations for trials and to predict risk before the phenotype of the disorder is manifest. Key components for future development of the pharmacogenomics of psychiatric disorders include understanding the mechanism of drug action, identification of candidate genes and their variants, and well-conducted clinical trials. Pharmacogenomic studies on AD, depression, and schizophrenia are briefly reviewed here as examples.

Pharmacogenomics of AD

AD is a polygenic disorder and several genes as well as polymorphisms are being identified. Their role as a risk factor and their relation to certain forms of the disease are under investigation. Although the cause of AD is unknown, a proportion of patients have autosomal dominant transmission (familial AD) and at least three genes are associated with this early onset form of the disease: those encoding β-amyloid precursor protein, presenilin 1, or presenilin 2. The majority of patients of any age have sporadic (nonfamilial) disease in which no mutation in the β*APP* or presenilin genes has been identified. However, another genetic risk factor, variants of *ApoE*, the gene that encodes apolipoprotein E, a constituent of the low-density lipoprotein particle, has been associated with AD. ε4 allele of the gene encoding apolipoprotein confers a significant risk for commonest, late onset sporadic form of the disease. Yet nearly one-quarter of Americans have one copy of the ε4 variation on the ApoE gene, meaning that they are at triple the average risk of AD. An additional 2% of Americans have two copies of ε4, putting them at 12 times the average risk of getting AD. This allele is the primary target for AD disease-related pharmacogenomic studies.

Cyp46, the gene encoding CYP46 enzyme, is a member of the cytochrome 450 family of enzymes and converts cholesterol to 24-hydroxycholesterol (24-OHC). Cyp46 is expressed exclusively in the brain and plays a key role in the hydroxylation of cholesterol and mediates its removal from the brain. Cyp46 influences brain Aβ load, cerebrospinal fluid levels of Aβ peptides, and phosphorylated tau. This study also observed a link between polymorphisms in Cyp46 gene and the genetic risk of

late-onset AD (LOAD). Subjects with the TT rs754203 polymorphisms in the Cyp46 gene exhibited a threefold increase in plaque load (a measure of aggregated Aβ) and a 37.5% increase in the CSF Aβ42. This risk was additive with that of ApoEε4. Subjects who had both Cyp46 and ApoEε4 polymorphisms had an odds ratio of 9.6 for AD compared with 4.4 for subjects with ApoEε4 and 2.2 for subjects with Cyp46. These results suggest an interaction between Cyp46 and ApoEε4 and indicate a link between cholesterol metabolism and AD because both genes regulate cholesterol metabolism.

Pharmacogenomics of Depression

Antidepressant treatment represents an ideal target for pharmacogenomics. Depression is a common disorder affecting over 10% of the North American population. If inadequately treated, depression can result in suicide, a common cause of death. Treatment for depression is expensive and protracted, and there are no biomarkers of treatment response. The identification of genomic markers of treatment response would constitute an enormous clinical advance of public health importance. Moreover, pharmacogenomics may lead to the identification of targets for the development of novel and hopefully more efficacious drugs that have a favorable safety profile.

There is strong evidence that the gene 15q14 is implicated in bipolar disorder. However, there is some evidence that a different gene altogether (7q11.2) is associated with a positive response to lithium. This suggests a pharmacogenomic strategy focusing on the treatment-relevant gene as well as continued study of the etiology of the disorder.

Pharmacogenomics of Schizophrenia

The exact cause of schizophrenia is not known but a genetic component is recognized. Pharmacogenomic studies in schizophrenia are mainly retrospective and have focused primarily on clozapine and variants in candidate genes of dopamine and 5-HT systems. No prospective study, designed for pharmacogenomic analysis, has been conducted on clozapine-treated patients. The first candidate gene examined with regard to clozapine response was DRD4 gene which codes for dopamine D4 receptor with the assumption that clozapine has a higher affinity for the D4 receptor than for the D2 receptor. Other studies indicate that 5-HT mediated mechanisms play a central role in antipsychotic drug action and that clozapine has a relatively high affinity for 5-HT_{2A} receptor. Further studies have approached pharmacogenomics through non-receptor targets by examining drug disposition rather than binding profiles. So far variants related to clozapine metabolism have not been strongly associated with clinical response.

Identification of susceptibility genes is likely to provide valuable insights into the etiology and pathogenesis of schizophrenia. Improvements in genomic technologies have resulted in the implication of genes at several chromosomal loci with identification of genetic subtypes of schizophrenia. Genes linked to schizophrenia, are being identified. Drug discovery can now be based on working with novel targets known to be causally involved in the pathogenesis of the disease.

Summary

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. Pharmacogenomics, along with several new biotechnologies, impacts all stages of drug development, starting with discovery, and finally for stratification of patients in clinical trials. Pharmacological aspects of genomics of important therapeutic areas – oncogenomics, cardiogenomics and neurogenomics – are described.

Chapter 6
Role of 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 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 2009e). Application to development of personalized medicine will be discussed here briefly. The role of proteomics in drug development can be termed "pharmacoproteomics". Proteomics-based characterization of multifactorial diseases may help to match a particular target-based therapy to a particular marker in a subgroup of patients. The industrial sector is taking a lead in developing this area. Individualized therapy may be based on differential protein expression rather than genetic polymorphism.

Proteomics will have a great impact on diagnosis during the first decade of the twenty-first century. By the end of the 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 (Hood et al. 2004). This will have a major role in creating a predictive, preventive, and personalized approach to medicine

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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 to understand type 2 diabetes mellitus as the insulin gene may be normal and the disease may arise from an abnormality at any point in the complex 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 any useful therapy because the 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, clearing dead cells, and restricting the spread of intracellular pathogens. To understand the functions of phagosomes, systematic studies for identification of their proteins have been conducted using proteomic approaches. The systematic characterization of phagosome proteins provides new insights into phagosome functions and the protein or groups of proteins involved in and regulating these functions.

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. Multicolor fluorescence activated cell sorting (FACS) techniques combined with phosophospecific antibodies are available and enable 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. The disturbance of the 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 proteins 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 oftentimes result in only subtle protein-folding abnormalities. In the case of the cystic fibrosis transmembrane receptor (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

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 clinical trials to treat nephrogenic diabetes

insipidus, 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 are as follows.

Mutations of the GnRH (gonadotropin-releasing hormone) have been identified in patients with hypogonadotropic hypogonadism (HH) and these can be rescued with a GnRH peptidomimetic antagonist that acts as folding template, stabilizing (otherwise) misfolded GnRHR receptor mutants and thereby restoring function. The antagonist can be removed after the correctly folded protein reaches the cell surface enabling the receptor to function normally. 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. This approach provides therapeutic opportunities for HH and other disorders resulting from protein misfolding.

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 becomes toxic to the liver. Its depletion in the lung can cause emphysema via a failure to block an enzyme that hydrolyzes the connective tissue elastin. Clinical trials are being conducted with 4-phenylbutyric acid (PBA); a drug that has been shown to be effective on mice transgenic for the human alpha-1-AT gene. PBA has been safely administered to children with disorders of the urea cycle, and therefore can bypass early phases of the drug approval process.

Significance of Mitochondrial Proteome in Human Disease

Disorders, due to mutations in genes affecting mitochondrial protein synthesis, may erode the bioenergetic capacity of the tissues contributing to the senescence process in aging. Because mitochondrial dysfunction has been implicated in numerous diseases, such as cancer, Alzheimer's disease, and diabetes, it is probable that the identification of the majority of mitochondrial proteins will be a beneficial tool for developing drug and diagnostic targets for associated diseases.

Current research aims to identify every protein within the mitochondria. To do this, highly purified mitochondrial preparations are completely disassociated, and the liberated proteins then separated via several techniques in parallel. Once separated, individual proteins are then digested, and the fragments identified using mass spectrometry techniques. The goal of completely characterizing the entire mitochondrial proteome is greatly facilitated by the use of robotics and dedicated bioinformatics. Comparisons of the proteome between mitochondria from healthy

persons versus patients will help identify changes associated with the disease, and therefore suggest potential interventional strategies.

Amino acid sequence profiles have been constructed for the complete yeast mitochondrial proteome using Bayesian priors (conditional probabilities that allow the estimation of the likelihood of an event on the basis of prior occurrences of similar events). These have been used to develop methods for identifying and characterizing the context of protein mutations that give rise to human mitochondrial diseases. Because these profiles can assemble sets of taxonomically very diverse homologs, they enable identification of the structurally and/or functionally most critical sites in the proteins on the basis of the degree of sequence conservation. These profiles can also find distant homologs with determined three-dimensional structures that aid in the interpretation of effects of missense mutations. This approach has the potential for assisting in identifying new disease-related genes.

Proteomic Technologies for Drug Discovery and Development

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.

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, who may not respond to conventional combination chemotherapy.

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 enable 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 studies have already provided insights into the mechanisms of action of a wide range of substances, from metals to peroxisome proliferators. Toxicoproteomics can increase the speed and sensitivity of toxicological screening of drugs by identifying protein biomarkers of toxicity. 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 proteomic technologies 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 programs 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 Adsorption, Distribution, Metabolism, Excretion-toxicity (ADME-tox) properties to performance in actual clinical trials. Some examples of the application of proteomics to toxicology are given below.

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.

An overdose of acetaminophen causes acute hepatotoxicity in rodents and humans but the underlying mechanism remains unclear. However, experimental evidence strongly suggests that the activation of acetaminophen and subsequent formation of protein adducts are involved in hepatotoxicity. Two-dimensional (2D) protein databases of mouse liver have been constructed using proteomics technologies to investigate proteins affected by acetaminophen-induced hepatotoxicity. Changes in the protein level are studied by a comparison of the intensities of the corresponding spots on 2D gels. The expression levels of several proteins are modified due to treatment with acetaminophen. Many of the proteins that show changed expression levels are known to be involved in the regulation of mechanisms that are believed to drive acetaminophen-induced hepatotoxicity. The 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-methyl-glutaryl-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.

Proteomics, LDH (lactate dehydrogenase) release and mitochondrial respiration (WST-1 reduction assay) have been used to detect cytotoxicity, morphological evaluation, and for estimating the reliable and sensitive biomarkers by using rat primary hepatocytes exposed to the compounds (acetaminophen, amiodarone, tetracycline and carbon tetrachloride) that are known to induce hepatotoxicity (Kikkawa et al. 2005). It was concluded that the cytotoxicity was detected earlier by measuring WST-1 rather than by measuring LDH release because the reduction of mitochondrial respiration is an expression of earlier toxicity for cellular function, while the measured increase in the LDH release occurs after the failure of the cell membrane. Mitochondrial respiration ability is a useful parameter of cytotoxicity for in vitro hepatotoxicity screening, as cytotoxicity can be detected during the early stage of exposure. In addition to the conventional biomarkers, several protein biomarkers which relate to oxidative stress and metabolism-regulation were detected. Further comprehensive analysis of defined proteins would be necessary to estimate the more sensitive toxicology biomarker.

Nephrotoxicity. An example of dose-related nephrotoxicity is that caused by cyclosporine A which has proven beneficial effects in organ transplantation. Proteomic analysis using 2D GE 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. 2-DE 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. Neurotoxin-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 the disturbance of the tertiary structure and a loss of function of the neurons. The proteome and the functional determinants of its individual protein components are, therefore, likely targets of neurotoxin 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) and more recently developed approaches (e.g., two-hybrid systems, antibody arrays, protein chips, 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 neurotoxin exposure, can provide quantitative data regarding changes in corresponding expression levels and/or post-translational modifications that might be associated with neuron injury.

Application of Pharmacoproteomics in Personalized Medicine

The advantages of the application of pharmacoproteomics in personalized medicine are:

- Pharmacoproteomics is a more functional representation of patient-to-patient variation than that provided by genotyping.
- It includes the effects of post-translational modification; pharmacoproteomics connects the genotype with the phenotype.
- This approach may accelerate the drug development process, by classifying patients as responders and non-responders.

Summary

Proteomics, which indicates PROTEins expressed by a genOME and the systematic analysis of protein profiles of tissues, parallels the related field of genomics, and is an important part of the basics of personalized medicine. The role of proteomics in drug development is termed “pharmacoproteomics”. Individualized therapy may be based on differential protein expression rather than genetic polymorphism.

Combining the genomic with the proteomics information reveals a more dynamic picture of the disease process. Single cell proteomics may be useful for predicting the optimal treatment for individual patients. 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. Toxicoproteomics can increase the speed and sensitivity of toxicological screening of drugs by identifying protein biomarkers of toxicity.

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 being, the human metabolome is the set of all metabolites in a human being. In a systems biology approach, metabolomics provides a functional readout of changes determined by the 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 nuclear magnetic resonance (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 of 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.

Researchers at the University of Alberta (Edmonton, Canada), funded by Genome Canada, have completed the first draft of the human metabolome. They categorized 2,500 metabolites, 1,200 drugs, and 3,500 food components, which can be found in the human body. The metabolome has been gathered into the human metabolome database (HMDB), which will enable researchers to find out what metabolites are associated with which diseases, what the normal and abnormal concentrations are, where the metabolites are found or what genes are associated with which metabolites (Wishart et al. 2007). Application of metabolomics to diagnostics, drug research, and nutrition might be integral to improved health and personalized medicine (Hunter 2009).

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Metabolomics Bridges the Gap Between Genotype and Phenotype

In general, the phenotype is not necessarily predicted by the genotype. The gap between the genotype and the phenotype is spanned by many biochemical reactions, each with individual dependencies on various influences, including drugs, nutrition, and environmental factors. In this chain of biomolecules from the genes to the phenotype, metabolites are the quantifiable molecules with the closest link to the 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.

Metabolomics provides the capability to analyze large arrays of metabolites for extracting biochemical information that reflects true functional end-points of overt biological events whereas other functional genomics technologies such as transcriptomics and proteomics merely indicate the potential cause for phenotypic response. Therefore they cannot necessarily predict drug effects, toxicological response, or disease states at the phenotype level unless functional validation is added.

Metabolomics bridges this information gap by depicting, in particular, such functional information because metabolite differences in biological fluids and tissues provide the closest link to the various phenotypic responses. Such changes in the biochemical phenotype are of direct interest to pharmaceutical, biotech, and health industries once appropriate technology allows the cost-efficient mining and integration of this information.

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 single nucleotide polymorphism (SNPs) and serum concentrations of endogenous organic compounds in 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.

The 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 and 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.

Metabolomics, Biomarkers and Personalized Medicine

Metabolomics has been used to identify biomarkers for disease and to identify off-target side effects in marketed drugs and new chemical entities in development. Compared to 25,000 genes and approximately a 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-based technologies, integration of 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. It is hoped that metabolomic profiling would be included in personalized medicine.

Metabolomic Technologies

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 the 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 liquid chromatography-mass spectrometry LC-MS technology achieve a lower sample throughput but provide unassailable identification and quantification of individual compounds in a

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.

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 because of 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 in the 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 mass spectral deconvolution and a broad linear dynamic range represent indispensable prerequisites for high quality spectra and peak shapes. Modern GC-TOF-MS applications and incorporated mass spectral 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
- Ability to analyze multiple pathways and arrays of metabolites simultaneously from microliter sample quantities

Urinary Profiling by Capillary Electrophoresis

Metabolomic approaches have become particularly important for the discovery of biomarkers in urine. The analytical technology for urine 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 urine 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 over 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 Automated Comparison of Electropherograms (ACE) 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 the 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 "-omic" 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 the findings are valid. Metabolomics is not immune to this problem but, when practiced effectively, the technology can reliably produce information 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 us to emulate the simultaneous acquisition of an unlimited number of precursors and neutral loss scans in a single analysis (Schwudke et al. 2006). This approach takes full advantage of rich fragment patterns in tandem mass spectra 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 owing 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 (Weckwerth and Morgenthal 2005). 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 interindividual 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.

A 'pharmacometabonomic' approach to personalizing drug treatment, developed by scientists at the Imperial College London in collaboration with Pfizer, uses a combination of pre-dose metabolite profiling and chemometrics to model and predict the responses of individual subjects (Clayton et al. 2006). A proof-of-principle

for this new approach, which is sensitive to both genetic and environmental influences, is provided with a study of paracetamol (acetaminophen) administered to rats. Predose prediction of an aspect of the urinary drug metabolite profile and an association between predose urinary composition and the extent of liver damage sustained after paracetamol administration was shown. The new approach, if successful, requires the analysis of the metabolite profiles of an individual from a urine, or other biofluid, sample. This new technique is potentially of great importance for the future of healthcare and the pharmaceutical industry and for the development of personalized medicine. The new method is expected to be synergistic with existing pharmacogenomic approaches. Pharmacometabonomics is in the early stage of development and will be studied in humans to evaluate its possible clinical application. Pharmacometabonomics could be used to preselect volunteers at key stages of the clinical drug development process. 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 (Haselden and Nicholls 2006).

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 after collection 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

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 the 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 a 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 makes it possible to assess 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. The American Society for Nutritional Sciences (ASNS) should take action to ensure that appropriate technologies are developed and that metabolic databases are constructed with the right inputs and organization. ASNS also should consider the social implications of these advances and plan for their appropriate utilization.

Summary

Whereas the human genome is the set of all genes in a human being, the human metabolome is the set of all metabolites in a human being. Metabolomics bridges the gap between the genotype and the phenotype and is an important basis of personalized medicine. Metabolomics has been used to identify biomarkers for disease and to identify the effects of drugs. Various metabolomic technologies include nuclear magnetic resonance, GC, and mass spectrometry. Pharmacometabonomic approach to personalizing drug treatment uses a combination of pre-dose metabolite profiling and chemometrics to model and predict the responses of individual subjects. Metabolomics/metabonomics also have a role to play in assessing drug toxicity and in guiding nutrition.

Chapter 8
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 the 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 have been used to provide individualization of therapy.

Recombinant Human Proteins

The number of therapeutic proteins approved for clinical use is increasing 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, quantification, and characterization of antibody responses are necessary. Immune response to therapeutic proteins in conventional animal models, predictive of the response in humans, has not been, except in rare cases. In recent years there has been a considerable progress in the 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 have already been applied in the early 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 recombinant proteins in combination with diagnostic tests to limit their use to patients in whom they are least likely to induce immune reactions.

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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 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 be hepatotoxic and are involved in drug-drug interactions. They may interfere with CYP450. 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 humanizing monoclonal antibodies (MAbs) has greatly advanced their utility in molecular targeting therapies. These will be described in more detail in the chapter on personalized cancer therapy. Several molecular biological and immunological studies have revealed the targeting properties of the host immune system and the biological mechanism 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 antibody designs and improved understanding of the mode of action of current antibodies 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 cell 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.

Various cells including stem cells, technologies, and applications are described in detail in a special report on this topic (Jain 2009h).

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 some lost functions of organ, i.e., organ repair instead of organ replacement. There are several problems associated with transplantation including organ rejection and currently most of the organ transplants are supported with 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

The term "stem cells" is applied to those 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. 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.

Role of Stem Cells Derived from Unfertilized Embryos

Using unfertilized human oocytes as a source for stem cell derivation 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 major histocompatibility complex (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 cancer progression biomarkers and, perhaps, cancer prevention drugs. Furthermore, these homozygous stem cells could be used in facilitating linkage studies and in verifying the function of a single nucleotide polymorphism (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 that 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, or heart tissue. This would be considered personalized cell therapy.

Use of Stem Cells for Drug Testing

With the ability to isolate, expand and study mesenchymal stem cells (MSCs) in vitro, an 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 the 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 1998b; Jain 2009i). It has three components; (1) identification of the gene that is mutated in the disease to obtain 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 individualized therapy. Another approach to personalizing gene therapy 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. This method has been applied to colon tumor dataset and some patterns, consisting of one or more genes, were found to reach a high frequency – 90%, or even 100%. Thus, they nearly or fully dominate one class of cells, even though they rarely occur in the other class. The discovered patterns were used to classify new cells with a higher accuracy than other reported methods. Based on these patterns, one can consider the feasibility a personalized treatment plan which converts colon tumor cells into normal cells by modulating the expression levels of a few genes.

Personalized Vaccines

The next era in vaccines will be ushered in by the new science of vaccinomics, which will enable the development of personalized vaccines, based on our increasing understanding of immune response phenotype/genotype information. Two important areas for application of personalized vaccines are viral infections and cancer.

Personalized Vaccines for Viral Diseases

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 human leukocyte antigen (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 (Poland et al. 2008). In addition, newly available data suggest that some vaccine-related adverse events may also be genetically determined and, therefore, predictable.

Personalized Cancer Vaccines

Personalized cancer vaccines can be patient-specific or antigen-specific. Examples of these are given here.

Patient-Specific Cancer Vaccines

This approach may generate an antigen-specific response even when the tumor antigens are not known. A cell therapy product is created using a technique that fuses the patient's own tumor cells with powerful, immune-stimulating dendritic cells (DC). 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.

Clinical trials of the patient-specific cancer vaccine in breast cancer, melanoma, and kidney cancer have demonstrated clinical or immunologic responses. The combined data from these studies show the ability of fusion vaccines to spark measurable responses in patients with advanced cancers. Together, the chemical fusion and electrofusion trials will provide a basis of comparison in multiple indications and will help guide further clinical development of the patient-specific vaccines. Patient-specific vaccines using this approach are in commercial development.

OncoVax (Intracel Corp, Frederick, MD), a patient-specific active immunotherapy, has been granted a special protocol assessment for the execution of a confirmatory phase III trial in stage II colon carcinoma patients. If successfully completed, the pivotal study, could be expected to form the basis of a biological license application. Intracel's previously randomized study demonstrated a statistically significant 33% increase in overall survival and a 40% reduction in deaths or recurrences in treated colon cancer patients compared to controls at 5 years.

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. As such, MyVax® is commonly referred to as personalized immunotherapy or personalized cancer vaccine. MyVax® Personalized Immunotherapy 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. MyVax® Personalized Immunotherapy is currently in a pivotal phase III trial and additional phase II trials for the treatment of B-cell non-Hodgkin's lymphoma.

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. DCVax®-Prostate is in a phase III clinical trial. Data from a phase I/II clinical trial support the overall safety of DCVax®-Prostate, and suggest that it may

induce an immune response. Clinical data obtained in this trial also suggest delayed times to progression of disease, especially in patients with no metastatic disease at entry. DCVax®-Brain has been granted an Orphan Drug designation and is in a phase II clinical trial for glioblastoma multiforme. DCVax-Lung has received clearance from the FDA for phase I trials.

Antigen-Specific Vaccines

Currently the scope of cancer immunization is limited because most of the vaccines have targeted antigens that are restricted to a subset of patients. This fits in with the concept of personalized medicine. 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.

If genes are identified in the majority of all cancers, a more universal approach to cancer vaccines can be considered. Success with these strategies will greatly depend on whether it is possible to induce robust immunity against the antigens identified, whether technical and regulatory issues of patient-tailored approaches can be adequately addressed, and certainly also which approach will be economically more advantageous. Currently, the universal approach appears to be unrealistic and even if it becomes feasible, it may not improve the management of cancer.

Autologous Cell Vaccines

An autologous cell vaccine is being developed by AVAX Inc. After removal of a patient's malignant tumor, cancer cells are treated with dinitrophenyl (DNP), a chemical compound known as a hapten, which binds to molecules on the surface of cells and helps trigger immune responses. DNP-treated cancer cells are combined with an adjuvant that enhances their effectiveness and are injected back into the patient. 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 easy to identify as foreign to the immune system has been well documented over the past 30 years. AC Vaccine technology applies this same process to cancer cell proteins and other molecules, using the patient's immune system to help prevent recurrence and increase the long-term survival rate.

The BIOVAXID™ (Accentia BioPharmaceuticals) cancer vaccine evokes the power of each patient's immune system and primes it to recognize and eliminate cancerous 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 the individual patient's cancerous cells, the vaccine is a true targeted, personalized therapy. The vaccine's anticancer effect is different from 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. It is being tested in phase III clinical trials at M. D. Anderson Cancer Center (Houston, TX) for follicular lymphoma, a form of non-Hodgkin lymphoma.

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. This fundamental property requires that each patient's immune system be trained to specifically recognize that patient's specific cancer. Based on this basic fact, Antigenics Corporation manufactures its cancer immunotherapeutic from each patient's own tumor tissue.

AG858 (Antigenics Inc) consists of autologous heat shock protein 70 (HSP70)-peptide complexes purified from the peripheral blood mononuclear cells of chronic myelogenous leukemia (CML) patients. HSPs shuttle peptides from one compartment of the cell to another. If the contents of the cell spill into the extracellular environment, during necrosis for example, HSPs send out a danger signal, basically recruiting antigen-presenting cells (APCs), such as DCs, which internalize the HSP-peptide complexes. There is evidence that when APCs take up HSPs together with the peptides they chaperone, the accompanying peptides are delivered into the antigen-processing pathways, leading to peptide presentation by MHC molecules. When DCs travel to the lymph nodes, T cells recognize the antigenic peptides and are specifically activated against cancer cells bearing these peptides. This personalized, therapeutic vaccine has been shown to eliminate cancer in a phase I clinical study of patients with CML who were also being treated with imitanib but had residual disease (Li et al. 2005).

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. Using mass spectrometry, several different MHC ligands can be characterized from the same tumor sample: derived from overexpressed gene products, proto-oncogenes, and frameshift mutations. By combining these two analytic

tools, it is possible to propose several candidates for peptide-based immunotherapy. This novel integrated functional genomics approach can be used for the design of antitumor vaccines tailored to suit the needs of each patient.

Personalized Melanoma Vaccines

Melacine melanoma vaccine (Corixa Corporation) consists of lysed (broken) cells from two human melanoma cell lines combined with Corixa's proprietary Detox™ adjuvant. Detox adjuvant includes MPL® adjuvant (monophosphoryl lipid A) and mycobacterial cell wall skeleton, both of which activate the human immune system in the context of vaccination. Melacine vaccine is approved in Canada and is administered as a two-shot vaccination delivered in four 6-month cycles, each consisting of 10 treatments followed by a 3-week rest. Patients who respond are maintained on long-term therapy.

The approval is pending in the US as further clinical trials have been conducted. 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. If the FDA approves Melacine for certain genotypes, it could become one of the first cancer vaccines in the US to be considered solely for patients with certain gene types, a sort of personalized vaccine.

A true personalized vaccine will be one in which patient's own cells are used. One clinical trial is using a vaccine which fuses the patient's own melanoma cells with their own DCs, which help the immune system to recognize cancer cells, to create a treatment designed to eradicate the patient's specific melanoma. The data from animal studies are convincing as melanoma was cured in nearly every mouse treated by this approach.

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 a 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 2009j). RNAi has been shown to control tumour cell growth in vitro. siRNA or plasmids expressing sequences processed to siRNA could provide an exciting new therapeutic modality for treating cancer. A siRNA targeting system is being used 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 the site of loss of heterozygosity. RNAi approach using oligonucleotide-based drugs may provide the required selectivity for ASI therapeutic approach. siRNA possesses unique characteristics which imply that siRNA can not only be used as a tool to study gene function, but might also be used as a genotype-specific drug to mediate ASI. RNAi may play an important role in personalized medicine. A few siRNAs are already in clinical trials. The role of RNAi in the development of personalized medicine is shown in Fig. 8.1.

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

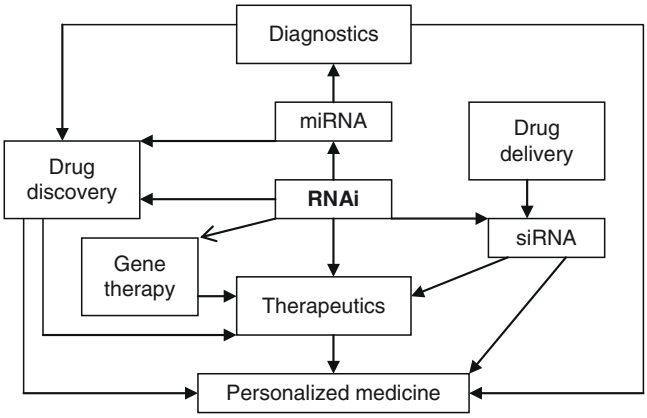

Fig. 8.1 The role of RNAi in the development of personalized medicine. © Jain PharmaBiotech

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 powerful tools 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. MicroRNA pharmacogenomics can be defined as the study of microRNAs and polymorphisms affecting microRNA 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. 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.

Summary

Examples of biological therapies are vaccines, MAbs, cell/gene therapy, and RNAi. These are particularly suitable for personalization. Vaccines made from the patient's own tumor cells are personalized therapies. Another example of personalized biological therapy is when adult stem cells from a patient are transformed into specialized cells for the treatment of a disease in the same patient.

Chapter 9
Development of Personalized Medicine

Introduction

In conventional medical practice, physicians rely on their personal experience in treating patients. In spite of advances in basic medical sciences and the introduction of new technologies, physicians continue to rely on their judgment and sometimes intuition because the practice of medicine is an art as well as a science.

Physicians of the last generation had limited access to information. With advances in molecular biology and its impact on medicine, a 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 a large number of studies with stratification of a large number of patients will have to be analyzed to make decisions about treatment for an individual. The massive amount of publications needs to be sorted out and analyzed for its relevance to individualized treatment.

The development of personalized therapy requires the integration of various segments of clinical medicine, pharmacology and biotechnology. Genotyping is an important part of such a system. Various technologies for genotyping have been described in the following chapter and their advantages as well as limitations have been pointed out. The vast majority of relevant gene variants are rare, making it difficult to demonstrate utility – in particular for the much more frequent heterozygous carriers who have only one affected allele. Moreover, multiple factors play a role such that genetic data represent only a portion of the information needed for effective therapeutic decisions. Therapeutic areas in which personalized medicine is expected to play an important role are listed in Table 9.1.

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Table 9.1 Important therapeutic areas for personalized medicine

Cancer
Cardiovascular disorders
Congestive heart failure
Hyperlipidemia
Hypertension
Inflammatory disorders
Asthma
Inflammatory bowel disease
Rheumatoid arthritis
Neurological disorders
Alzheimer's disease
Epilepsy
Parkinson's disease
Pain management
Psychiatric disorders
Schizophrenia
Depression
Viral infections
Hepatitis C virus
HIV
Miscellaneous Disorders
Hormone replacement therapy
Organ transplants
Renal disorders
Smoking cessation
Trauma and burns

Non-genomic Factors in the Development of Personalized Medicine

Although personalized medicine is supposed to be based mostly on pharmacogenomics, a number of other factors that vary among individuals are taken into consideration. Metabolomics was described in Chapter 7. Other factors are discussed briefly in this chapter.

Personalized Medicine Based on Circadian Rhythms

Diverse physiological and metabolic processes exhibit circadian rhythms, which are endogenous self-sustained oscillations within a period of ~24 h. They are coordinated by a biological clock situated in the suprachiasmatic nuclei 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.

The rhythms of disease and pharmacology can be taken into account to modulate treatment over the 24 h period, and is known as 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. The half-life of a drug can vary as a function of the hour of administration.

The efficacy and 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" has been composed consisting of >100 "time-indicating genes," whose gene expression levels can represent internal BT (Ueda et al. 2004). The power of this method was demonstrated by the sensitive and accurate detection of BT and the sensitive diagnosis of rhythm disorders. These results demonstrate the feasibility of BT detection based on single-time-point sampling, suggest the potential for expression-based diagnosis of rhythm disorders, and may translate functional genomics into chronotherapy and personalized medicine.

Intestinal Microflora

Gut Microbiome Compared to Human Genome

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 2,062 PCR-amplified 16 S 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 superorganisms 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 is called metagenomics. This has implications for clinical diagnosis and the 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 be consistent with the optimal health of an individual. Such an analysis would also identify bacteria that are resistant to certain antibiotics, and enable the 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 the 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, 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 pharmaceutical companies are developing a metabonomic technology that will identify 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.

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.

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 by tomography, 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.

Personalized Approach to Clinical Trials

Use of Bayesian Approach in Clinical Trials

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. The 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 the Bayesian approach, in contrast to the standard approach, the trial design exploits the results as the trial is ongoing and is adapted based on these interim results. In order to have 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 greatly on its acceptance by the FDA in determining the safety and efficacy of new treatments. The Food and Drug Administration of USA (FDA) has already approved the drug Pravigard Pac (Bristol-Myers Squibb) for the prevention of secondary cardiac events based on data evaluated using the Bayesian approach.

Individualizing Risks and Benefits in Clinical Trials

One study has comprehensively reviewed the basic and clinical evidence that explains how drugs like rofecoxib, celecoxib, and valdecoxib confer a small, but absolute, risk of heart attack and stroke (Grosser et al. 2006). 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 non-steroidal antiinflammatory drugs (NSAIDs); (b) whether adjuvant therapies, such as low-dose aspirin, will mitigate the hazard and if so, at what cost; (c) whether cyclooxygenase-2 (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.

Another study builds on the theme of individualized therapy, demonstrating a 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 that approximately 30% of the 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 the 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 the 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 Genetic Banking Systems and Databases

Genetic databases will be an important source of information for the development of personalized medicine. Most of these are covered under the term “biobanks”.

Role of Biobanks in the Development of Personalized Medicine

A biobank is a collection of biological samples and associated clinical data. There are biobanks for diagnostics as well as therapeutics. With the advent of the genomic era, the traditional purpose of biobanks, such as blood banks, for the storage and distribution of blood, has not been expanded 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. Some of the proposed or operational biobanks in the public, private and academic sectors are shown in Table 9.2.

Table 9.2 Biobanks relevant to personalized medicine

Name of biobank	Web site	Function
CARTaGENE (Quebec, Canada)	www.cartagene.qc.ca/	See text for details
deCODE Genetics	www.decode.com	Secure Robotized Sample Vault: for banking genetic samples of 100,000 Icelanders linked to Icelandic Health Database and genealogical records
Estonian Genome Project	www.geenivaramu.ee	Government effort to establish a national genetic/medical database of one million volunteers
Genomic Research in the African Diaspora	www.genomecenter.howard.edu	Howard University project to collect DNA and health information from 25,000 Americans of African descent
Karolinska Institute (Stockholm, Sweden)	http://ki.se/kiBiobank	Swedish academic bank collecting human biological material for molecular and genetic research
UK Biobank	www.ukbiobank.ac.uk	Government plan to collect genetic samples from 500,000 volunteers between the ages of 45 and 69
EU Biobanking	www.biobanks.eu	See text for details

UK Biobank

The UK Biobank project will be the world's biggest 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 their 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. UK Biobank published a Science Protocol for public comment in 2005. Following ethical approval, pilot studies commenced in 2006.

Biobanking and Development of Personalized Medicine in the EU

The Biobanking and Biomolecular Research Infrastructure (BBMRI, www.biobanks.eu), which started the preparatory phase in February 2008, will pool all

the information 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, and 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 the 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 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 the use of the patient data to acquire deeper insights into the causes of disease.

CARTaGENE for Biobanks in Canada

In 2007, the Canadian government and the government of Québec announced a grant of CA$34.5 million (US $31.9 million) for 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.

Personalized Medicine Based on PhysioGenomics™ Technology

PhysioGenomics™ (Genomas Inc.) is a proprietary technology based on systems biology, which rapidly analyzes multiple genes and baseline determinants of environmental responses for an individual. This technology unravels preexisting genetic (inherited DNA variability) and physiological determinants of response to each intervention, be it exercise, diet or drug (Ruano et al. 2006).

PhysioGenomics integrates genotypic and phenotypic measures to analyze variability among individuals within a population. Genotypes and physiological or clinical phenotypes are analyzed to discover statistical associations to environmental responses in individuals similarly exposed or challenged, to exercise, diet or drugs. Variability in a genomic marker among individuals that tracks with the variability in the quantitative response establishes associations and possible mechanistic links with specific genes. PhysioGenomics integrates the engineering systems approach with molecular probes stemming from genomic markers available from industrial technologies and the Human Genome Project. The strategy of "predict response and intervene" is quite distinctive from pure gene discovery for disease diagnosis. PhysioGenomics marks the entry of genomics into systems biology. The unintended and largely poorly understood effects of exercise, diet, and drugs are multicomponent interventions suitable for PhysioGenomics and systems biology.

The gene variability, measured by single nucleotide polymorphism (SNPs), is correlated to the physiological responses of a population, or the output. PhysioGenomics technology determines how the SNP frequency varies among individuals similarly responding to the input over the entire range of the response distribution. The unintended and poorly understood mechanisms of adverse drug reaction (ADR's) involve multiple physiological pathways suitable for PhysioGenomics analysis. The medical management products derived from PhysioGenomics technology is termed "PhyzioType™ Systems" (Genomas Inc), which is PhyzioType™ is used to predict responses to diet, exercise and drug treatments, and to select the best treatment for the patient from these options. It is a novel product in healthcare for guiding treatment based on unique integration of existing modes of medical management with genetic information on treatment responses. In a fundamental way the PhyzioType™ seamlessly combines "nurture", how the patient presents in middle age with decades worth of environmental, cultural and life-style influences on his own health, with "nature", the patient's genetic constitution inherited at birth.

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, the detection of SNPs, and proteomic data would require bioinformatic tools for cataloguing and analysing the 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 9.3.

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

Table 9.3 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
Applications in organization of personalized medicine
Personalized prognosis of disease
Linking patient-specific and knowledge-based information
Linking patient medical records and genetic information

classification. Classification can predict clinical outcome based on the chosen features. Computational diagnostics includes the 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 for 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 through current efforts in promoting the research, education and awareness of the upcoming integrated inter/multidisciplinary field (Yang et al. 2008).

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 (Fierz 2004). 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 medical record, 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 doctors and hospitals. They can improve the quality and safety of patient care by reducing errors in prescriptions. 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 many medical centers across the US are spending 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 the majority of US patients are treated, lagged behind in adopting EHRs because of the

high initial costs involved and the need for support and training. Only 13% of US physicians have a basic EHR system and 4% report having an extensive, fully functional EHR system (DesRoches et al. 2008). Financial barriers are viewed as having the greatest effect on decisions about the adoption of EHR.

To improve this situation, the Taconic Health Information Network in New York State is introducing an affordable and practical system for computerization of patient records in small medical practices. Although many technical problems need to be resolved EHRs are touted for their ability to reduce medication errors and redundant procedures while improving diagnostic accuracy and facilitating electronic prescribing. All these lead to the reduction of healthcare costs while improving patient care. EHRs can trim costs from the US national healthcare budget for those who suffer from one or more of four or five diseases that produce 75% of healthcare costs: diabetes mellitus, asthma, congestive heart failure and coronary artery disease.

In 2007, the National Human Genome Research Institute (NHGRI) announced plans to fund the development of methods and procedures for using EHRs in genome-wide studies that rely on biorepositories. NHGRI will issue a request for applications in 2007 that will fund groups affiliated with existing biorepositories to develop methods and procedures for genome-wide studies in participants with phenotypes and environmental exposures defined by electronic medical records, 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 (Robson and Mushlin 2004). 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. GMS is being developed at IBM R&D Labs (Haifa, Israel). 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 the use of micro arrays need to be integrated with existing medical records and then be made readily accessible to the practicing physician in a standardized format that enables information from one patient to 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 micro array 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.

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 data points (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.

Personalized Prognosis of Disease

Genomic and clinical data have been combined for personalized prediction in disease outcome studies. A typical 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

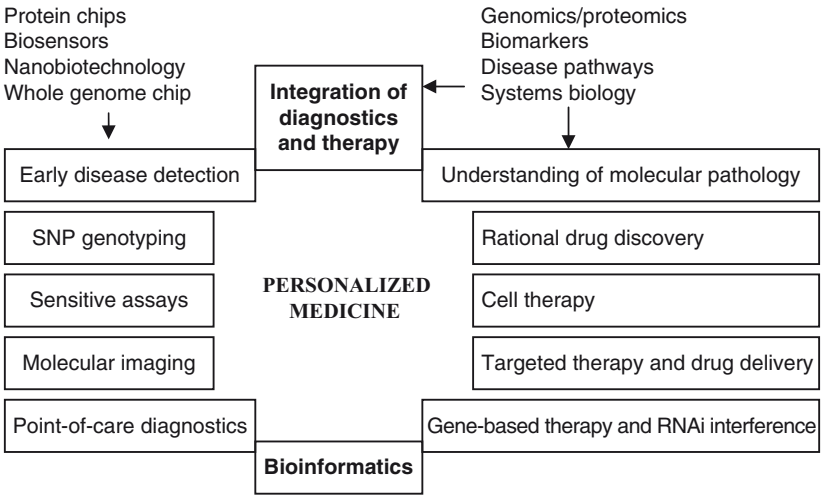

Fig. 9.1 Integration of technologies for the development of personalized medicine. © Jain Pharma-Biotech

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.

Integration of Technologies for Development of 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. 9.1.

Summary

This chapter deals with various factors that influence the effect of drugs and should be taken into consideration for the development of personalized medicine. These include chronobiology and metabolic interactions of the host and the intestinal microflora. Drug delivery and molecular imaging are also important considerations. Clinical trials involving personalized therapies require special methods and statistical approaches. Other important issues concern biobanking, bioinformatics and electronic records for implementation of a personalized healthcare system. Finally integration of several technologies is an important feature for developing personalized medicine.

Chapter 10
Personalized Therapy for Cancer

Introduction

Management of cancer has been unsatisfactory in the past but an understanding of the molecular, genetic and genomic aspects of cancer started to accelerate progress in cancer therapy (Jain 2005). 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. The role of oncoproteomics in the 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 chapter describes 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, known as transcriptional profiling.

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Table 10.1 Factors that drive the development of personalized therapy in cancer

Progress in pathophysiology of cancer
Advances in application of proteomic technologies in cancer
Transcriptional profiling in cancer
Molecular diagnosis of cancer is advancing rapidly
Advances in cancer vaccine technologies
Cancer biomarkers can be used for diagnosis as well as drug targets
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 million to 2.6 million)
Search for better treatments due to limited efficacy and toxicity of chemotherapy
Incentive to development from motivated physicians, patients and third party payers
Examples of personalized treatment of cancer are already in practice

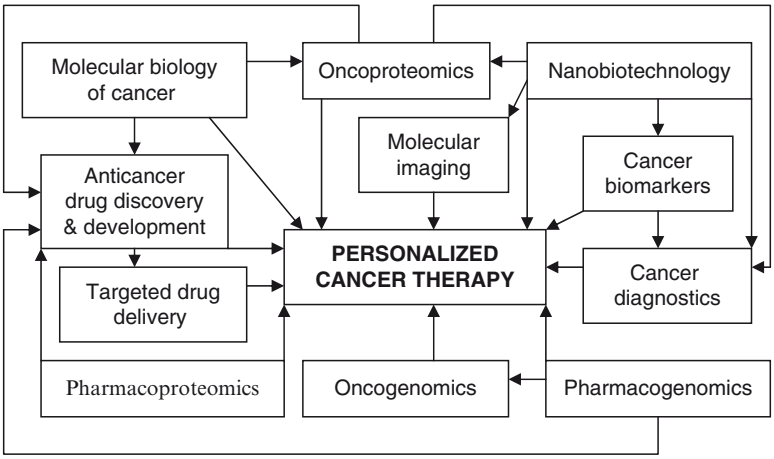

Fig. 10.1 Relationships of technologies for personalized management of cancer. © Jain PharmaBiotech

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.

Impact of Molecular Diagnostics on the Management of Cancer

Molecular diagnostics influences cancer management in several ways that lead 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.

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 has been developed to target specific

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 MRD
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

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 biomarkers. Gene expression profiling technologies such as differential analysis of transcripts with alternative splicing (DATA) 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 to become 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 is available for identifying alterations to chromosomes that occur when cells become malignant. Genomic 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. This approach has revealed many previously unknown additions and deletions in human breast cancer cells. 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. There are two general approaches. In an unsupervised approach, pattern-recognition algorithms are used to identify subgroups of tumors that have related gene-expression profiles. In a supervised approach, 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 biomarkers 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 in an affected individual. Clinically relevant subclasses of bladder carcinoma have been identified using expression microarray analysis of well-characterized bladder tumors. A classifier based on this analysis has provided 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.

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 (PD) endpoints and prognostic biomarkers for predicting outcome and patient selection. Expression profiling can be used alongside gene knockout or knockdown methods such as RNA interference (RNAi).

Detection of Loss of Heterozygosity (LOH)

Many cancers are characterized by chromosomal aberrations that may be predictive of disease outcome. Human neuroblastomas are characterized by 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 gene chip can be customized to assess region-specific LOH by genotyping multiple single nucleotide polymorphisms (SNPs) simultaneously in DNA from tumor tissues. 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 microarrays directly identify 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.

Diagnosis of Cancer of an Unknown Primary

Metastatic cancer of unknown primary site (CUP) accounts for approximately 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) has 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 (PBC) 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. Pathwork Informatics Inc is developing diagnostics for CUP using microarrays and gene expression analysis.

Diagnostics for Detection of Minimal Residual Disease (MRD)

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 “MRD”. Reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to detect BCR-ABL transcripts in chronic myeloid leukemia (CML) in a chronic phase. Patients who attain a molecularly defined minimal tumor burden have a higher rate of progression-free survival than those who do not. Such molecular data thus provide support for the position of imatinib as the drug of choice in CML.

Fluorescent In Situ Hybridization

Fluorescence in situ hybridization (FISH) is now used routinely in the clinical laboratory in every phase 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

Gene-expression profiling has been used to improve the design of cancer drugs that have shown some promise in clinical trials. 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 applications of this approach are given below:

- 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 (ALL), 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.
- A conserved BMI-1-driven pathway, which is similarly engaged in both normal stem cells and a highly malignant subset of human cancers diagnosed in a wide range of organs, uniformly exhibits a marked propensity toward metastatic dissemination as well as a high probability of unfavorable therapy outcome.

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 are 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 of prostate tumors has been done using immunohistochemistry 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 (Lapointe et al. 2004). In multivariate analysis, MUC1 and AZGP1 staining were strong predictors of tumor recurrence independent of tumor grade, stage, and preoperative prostate-specific antigen levels. These results 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 signatures that predict sensitivity to individual chemotherapeutic drugs have been developed by using in vitro drug sensitivity data coupled with microarray data (Potti et al. 2006). Many of these signatures can accurately predict clinical response in individuals treated with these 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 helped 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 using them in combination with existing targeted therapies.

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. Now, researchers in the Children's Hospital Informatics Program (CHIP) at the Harvard-MIT Division of Health Sciences and Technology report that a genetic profile indicating chromosomal instability – an increased tendency to develop chromosomal aberrations, critical in cancer development – is predictive of clinical outcome in a broad range of cancer types (Carter et al. 2006).

Chromosomal instability leads to a condition known as aneuploidy, in which chunks of DNA are either missing or duplicated. The technique indirectly measures the degree of aneuploidy and thus the degree of chromosomal instability by looking for abnormal expression levels of genes at the different chromosomal locations. The authors identified a 25-gene signature of chromosomal instability from specific genes whose expression was consistently correlated with total functional aneuploidy in several cancer types. 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 case of grade 1 and grade 2 breast cancers, distinguish the more aggressive cancers within each grade.

Using data on gene expression (activity) from 18 previous studies of cancer, representing six cancer types, they 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 for cancer.

Isolation and Characterization of Circulating Tumor Cells (CTCs)

Viable tumor-derived epithelial cells (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 the biology of metastasis. 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 the 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 the 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 non-small cell lung cancer (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 thxe biotransformation of anticancer agents in vivo. Variability between individuals in the pharmacokinetics (PK) 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 (CPM), 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.

Personalized Therapies Based on Oncogenic Pathway 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. Artificial cancer conditions can be created by introducing a series of oncogenes into otherwise normal cells. By comparing gene expression patterns in normal cells versus cells harboring oncogenes, it is possible to demonstrate 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 can identify patterns of pathway deregulation in tumors and clinically relevant associations with disease outcomes.

Clustering tumors based on pathway signatures further define 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 (Bild et al. 2006). 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, for example, then physicians could choose drugs that target only Myc and Ras. If the SRC and E2F3 pathways are highly active, then drugs that target these pathways can be selected. Because tumors arise from multiple defective genes and their malfunctioning proteins, their treatment 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 to the combination of drugs that 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.

Role of Molecular Imaging in Personalized Therapy of Cancer

Molecular imaging has markedly improved not only the diagnosis of cancer but also its management. It has enabled the combination of diagnosis with therapeutics. Some of the technologies are described here.

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. Now metabolic imaging with 18F fluorodeoxyglucose (FDG)-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) are being used increasingly in preclinical studies in animal models to document the effects of genetic alterations on cancer progression or metastases, the detection of MRD, 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 noninvasively image 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 (El-Deiry et al. 2006). 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 (RT)-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 tumor necrosis factor (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 PK and 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 PD evaluation of compounds.

Further efforts are needed in this area and the pharmaceutical industry needs 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 PD 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 antivascular agents, and gene expression with relevance to gene therapy. If an in vivo diagnostic is available to monitor the effects of the numerous available antiangiogenesis agents on tumors, it can help us to define responders and non-responders.

Molecular Imaging as Guide to Cancer Treatment

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, single-photon emission computed tomography (SPECT) and magnetic resonance

imaging (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 the 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 the 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 who are suited 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.

Functional Diffusion MRI

Functional diffusion MRI scan (Molecular Imaging Products) could help physicians decide quickly whether treatment for brain tumors is having any effect. The scan uses MRI to track the, or movement, of water through the brain (Moffat et al. 2005). Tumor cells block the flow of water, so 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 assessed whether the therapy was 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 has not, the management approach may be altered depending on the tumor. Speeding up this process can save patients from often-uncomfortable treatments that may also 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.

Role of FDG-PET/CT in Personalizing Cancer Treatment

Multimodality imaging, as represented by PET, has a definite role in the evaluation of a patient with cancer. Fluorodeoxyglucose-positron emission tomography (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 the 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 NSCLC, reduction of metabolic activity as demonstrated by FDG-PET after one cycle of chemotherapy is closely correlated with the final outcome of therapy (Weber et al. 2003). 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 imatinib. Preliminary studies show a marked decrease of FDG uptake in GIST tumors within 24 h in patients who go on to show clinical response to imatinib. 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 have been demonstrated by SPECT and provide a better understanding of the timing of radiolabeled annexin V uptake for its development as a marker of therapeutic efficacy (Mandl et al. 2004).

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).

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.

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 assemble with a heregulin-modified protein directed at the human Epidermal Growth Factor Receptor (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 did 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.

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 (CRC) (Sjoblom et al. 2006). Analysis of 13,023 genes in 11 breast and 11 CRCs revealed that individual tumors accumulate an average of approximately 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. Using microarray analysis to evaluate a previously established 70-gene prognosis profile, a series of consecutive patients with primary breast carcinomas have been classified as having a gene-expression signature associated with either a poor prognosis or a good prognosis. The gene-expression profile (tumor signature) is found to be a more powerful predictor of the outcome of disease in young patients with breast cancer than standard systems based on clinical and histological criteria. Currently, 70–80% of patients that receive adjuvant therapy would have survived without it, and chemotherapy has significant side effects and long-term consequences. This classification method can predict those that should receive treatment as effectively as other methods, while reducing the number who receive treatment unnecessarily. Gene signatures therefore seem to be the way forward in predicting outcome, and should pave the way for new therapies that are tailored for the patient.

Gene-expression profiles based on microarray analysis can be used to predict patient survival in early-stage lung adenocarcinomas. 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 progress 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.

Impact of Biomarkers on Management of Cancer

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.

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 quantification 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 the 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 who had primary breast tumors that were negative for HER-2/neu expression by immunohistochemistry. According to many immunohistochemistry (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 (L-ASP)Treatment of Cancer Guided by a Biomarker

L-ASP, a bacterial enzyme used to treat ALL, 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 fivefold. 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.

Determination of Response to Therapy

Several approaches have been investigated for predicting and monitoring response to anticancer chemotherapy. Some of these are described here.

Phenotype-Based Cell Culture Assays

Phenotype-based cell culture assays are used for predicting anticancer drug responses in individual cancer patients. These are based on the outgrowth and short-term primary culture of epithelial cells derived from pieces of solid tumors that are obtained at the time of tumor resection. The tumor cells are isolated and maintained in short-term culture before drug testing and their epithelial identity is verified by immunohistochemical staining methods. Cells are exposed to the anticancer drug. Using an automated image analysis system, cell kill is measured microscopically by counting the number of live cells remaining after dead cells have detached and are subsequently rinsed away.

Ex Vivo Testing of Tumor Biopsy for Chemotherapy Sensitivity

Assays are used to measure 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. They are not used for choosing a first-line 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 (Precision Therapeutics) 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 the anticancer agent Methylnitronitrosoguanidine (MNNG) with 94% accuracy. MNNG alkylates certain DNA bases, leading to mutagenesis. Some of this damage can be repaired by the DNA methyltransferase Methyl Guanine Methyl Transferase (MGMT). But if it is not, the DNA mismatch repair (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.

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.

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.

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 have identified protein patterns that correlated with tumor histology and patient survival (Schwartz et al. 2005). The protein patterns described served as an independent indicator of patient survival. These results show that this new 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 a real-time monitor 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, Food and Drug Administration (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 (CDDE) from Roche Diagnostics (Mannheim, Germany). 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 RT 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 (Holdenrieder et al. 2004). According to the same authors, prediction of efficacy of chemotherapy in NSCLC requires the following:

- Staging
- Age
- Baseline value of 1 CYFRA 21–1
- 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. 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 are used to image and quantify vascular effects of two different antitumor therapies in pancreatic tumor-bearing mice treated with anti-vascular vascular endothelial growth factor (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 to 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.

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. Cernostics Pathology is 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.

Targeted Cancer Therapies

Targeted cancer therapy means selective action against molecular targets expressed in tumors. Conventional small-molecular drugs are 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 therapeutics target the underlying defects in patient tumors, molecular diagnostics are required to identify patients with particular genetic aberrations in the pathway to enable personalized cancer treatment.

Functional Antibody-Based Personalized 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 biomarkers that distinguish a cancer cell from a normal cell, and one person's cancer from another's. The selection of antibodies may be based on their ability to activate an antigen to selectively produce cancer cell death. The antibodies are functional in and of themselves and are drug candidates at the very outset

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. One of

these, ARvitamab (ARIUS' ARH460–23), suppresses tumor growth with the following features: (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 nontoxic in animal models. The putative target antigen for ARvitamab exhibits increased expression in many cancers including those involving the breast, pancreas, colon and prostate, as well as nonepithelial cancers such as melanoma and lymphoma.

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.

Personalized Radiation Therapy

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 markers 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.

In 5% of patients radiation therapy treatment produces serious side effects. 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). With this tool, physicians may soon be able to tailor-make 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 RT 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.

Molecular Diagnostics Combined with Cancer Therapeutics

Cancer is a good example of a combination of diagnostics with therapeutics, which would be useful for personalized management of cancer. Examples of combined diagnosis and therapeutics for cancer are listed below and will be discussed further under personalized management of various cancers.

- Flow cytometry testing for MRD in CLL treated with alemtuzumab
- Abl mutations testing in CML for imitanib-resistance
- EGFR mutations testing in NSCLC for treatment with erlotinib) /gefitinib
- 5q FISH testing in myelodysplastic syndrome (MDS) for lenalidomide therapy

MAbs can be used both for diagnosing and targeting cancer. Some other therapies are in development and one of the examples is capecitabine (Roche's Xeloda) – a novel, oral fluoropyrimidine carbamate rationally designed to generate 5-fluorouracil (5-FU) preferentially in tumor tissue via a three-step enzymatic cascade. Roche is investigating diagnostic tests based on various biomarkers – thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase

(DPD – to predict responders to this therapy. Proof-of-principle studies for the biomarkers are running concomitantly with clinical trials of capecitabine.

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 have been developed as targeted therapeutics for the diagnosis, imaging, staging and treatment of cancers including those involving breast, bladder and stomach. 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 extensive potential for future development. Combinatorial libraries have been used for the selection of aptamers that bind to a well-characterized and established cancer marker 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.

Role of Nanobiotechnology in Personalized Management of Cancer

The role of nanobiotechnology in developing personalized approaches to the management of cancer has been described elsewhere (Jain 2005b, 2008). Nanodiagnostics have the potential to improve early diagnosis of cancer. Nanobiotechnologies will

also improve detection of cancer biomarkers as the basis for devising diagnostics as well as therapeutics. Some examples of the application of nanobiotechnology in improving cancer management are as follows.

αNβ3-targeted paramagnetic nanoparticles have been employed 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 the 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.

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 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. Serial analysis of gene expression (SAGE) studies have demonstrated that tumor and normal endothelium are distinct at the molecular level, a finding that may have significant implications for the development of antiangiogenic therapies.

One study has shown that 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 (Yu et al. 2001). Although it remains to be seen whether these findings translate to humans, the results suggest that those 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.

The 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 Q-PCR. The cancer clinical trial toolkit, including biomarkers that can detect antitumor activity of anticancer agents, can guide patient selection for specific drug treatments.

Screening for Personalized Anticancer Drugs

Several compounds are being screened for their ability to kill engineered carcinogenic cells but not their isogenic normal cell counterparts. Novel compounds with genotype-selective activity have been identified, including doxorubicin, daunorubicin, mitoxantrone, camptothecin, sangivamycin, echinomycin, bouvardin, and erastin. Screening assays have the potential to be used for finding the function of any given gene. The screen might be useful in identifying the drugs that are best suited to each patient's cancer, each with its own specific molecular profile.

Role of Epigenetics in Development of Personalized Cancer Therapies

In addition to having genetic causes, cancer is also an epigenetic disease. Epigenetics involves the study of chromatin modifications that affect gene expression without altering DNA nucleotide sequences such as in aberrant DNA methylation and histone acetylation. 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.

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. GenomicTree's MDScan™ technology for systematic and comprehensive genome-wide discovery of epigenetically silenced genes uses affinity-based methyl DNA enrichment, a bisulfite-free method, for selective enrichment of methylated DNA. The selectively isolated methyl DNA can be used for microarray analysis. This technology will lead to the discovery of novel methylation biomarkers for early detection of cancer, staging, risk of recurrence, and prediction of response to drug therapy.

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 tailor treatment strategies to individual patients. Most traditional anticancer agents primarily affect 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 anticancer 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 (Song and Miele 2007).

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 (Jain 2004). 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 200,000 (Chaurand et al. 2004). Laser-capture microdissection (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.

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 multicenter phase II trial of the proteasome inhibitor, bortezomib, in relapsed and refractory myeloma patients has revealed significant activity in a heavily pre-treated patient population and represents the first anticancer 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 LOH predicts poor therapeutic outcome in patients treated with RT alone. It also indicates that head and neck cancer patients with M6P/IGF2R allelic loss benefit most from chemotherapy added to RT.

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, a humanized MAb 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. ALL 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 is considered to occur 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 a 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 if 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 of this analysis and previous data from 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, if confirmed by other analyses of previous, well-designed clinical trials or by future prospective, randomized, controlled studies, these findings would 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 biomarkers 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. Pharmacogenetics, therefore, is particularly appealing for oncology. Cytotoxicity to chemotherapy agents 5-FU and docetaxel, which have distinct mechanisms of action, are heritable traits varying with dose. In cell lines, both these agents were shown to cause apoptotic cell death involving caspase-3 cleavage (Watters et al. 2004). The investigators rapidly found potential connections between these two chemotherapy drugs and two regions of human DNA that contain approximately 100 genes each. The initial test of the new approach found connections between increased sensitivity to the drugs and areas on chromosomes 5 and 9. This study identifies genomic regions likely to harbor genes important for chemotherapy cytotoxicity using genome-wide linkage analysis in human pedigrees and provides a widely applicable strategy for pharmacogenomic discovery without the requirement for a priori candidate gene selection. The potential application of this discovery is that patients whose cells are particularly sensitive to chemotherapy may be able to be treated with relatively low doses, reducing side effects. Patients whose cells are particularly resistant may need special or added medications to assure a good outcome.

Polymorphisms in TS, MTHFR, and FCGR3A, as well as the polymorphic DNA repair genes XPD and XRCC1, influence response to chemotherapy and survival outcomes. Fluorouracil's principal biochemical target, TS, shows wide variation of expression in normal and tumor cells. It has been investigated as a predictive factor for the efficacy of 5-FU. Retention of heterozygosity at one or more 17p or 18q sites was associated with the ability to benefit from adjuvant 5-FU. These results support the principle of developing molecular biomarkers as predictive factors in treatment decisions. Prospectively stratifying patients based on genotype may identify subpopulations likely to experience severe toxicity or those likely to benefit from a particular drug. Polymorphisms of CYP 1A2, thiopurine methyltransferase (TPMT), DPD, and UGT1A1, in relation to irinotecan therapy, are also important for the metabolism of anticancer drugs.

CYP1A2

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 inter-individual 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 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 enables physicians to predetermine patients' TPMT activity levels based on whether 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 ALL. 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

DPD is responsible for 80% of the degradation of 5-FU, 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-FU 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 for CRC 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. 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 irinotecan 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 PK parameters or the phenotypes of the enzymatic activity. 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 of natural history. A mechanism-based framework using cDNA arrays and computational models has promise in improving diagnosis and prediction, thereby making tailored therapy possible. Treatment strategies may be tailored to individuals based on tumor cell kinetics. A computational model of kinetically tailored treatment has been developed to predict drug combinations, doses, and schedules likely to be effective in reducing tumor size and prolonging patient life. The model incorporates intratumor heterogeneity and evolution of drug resistance, apoptotic rates, and cell division rates. This model 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 an anticancer drug development scheme 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 a crucial determining factor in its development. A multiscale mathematical model of cancer invasion, which considers cellular and

microenvironmental factors simultaneously and interactively can forecast how tumors grow and invade tissue (Anderson et al. 2006). The model simulations predict that harsh tumor microenvironment conditions (e.g. hypoxia, heterogenous extracellular matrix) exert a dramatic selective force on the tumor, which grows as an invasive mass with indistinct 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.

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).

Drug 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.

One explanation of development of resistance is that when cells become cancerous, they also become 100 times more likely than regular cells to genetically mutate. 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.

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 doxorubicin, paclitaxel, and many more anticancer drugs. A simple DNA test can enable a physician to predict drug uptake at the start 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.

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.

Pharmacogenetics and pharmacogenomics studies of the relationship between individual variations and drug response rates reveal that genetic polymorphisms of specific genes are 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. LOH at specific regions of chromosomes has been identified in specific cancers but its effect on treatment outcome remains controversial.

Detection of Drug Resistance in Cancer by Metabolic Profiling

Acquired resistance to imatinib mesylate is an increasing and continuing challenge in the treatment of BCR-ABL tyrosine kinase positive leukemias as well as GISTs. 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 (Serkova and Boros 2005). 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.

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).

A Systems Biology Approach to Drug Resistance in CRC

Mechanisms that may have important implications for drug efficacy and actively contribute to innate resistance in CRC are:

- High levels of TS, 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 be 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.

Management of Drug Resistance in Leukemia

Imatinib mesylate, an approved drug, causes remission in patients with CML. Despite these positive response rates, a subset of patients do not respond to therapy fully or at all, and approximately 4 to 5% of successfully treated patients annually develop resistance to imatinib during therapy with a recurrence of their disease manifestations. The molecular hallmark of CML is a mutation known as BCR-ABL. This mutation is the specific target for imatinib 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 imatinib 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 imatinib. Genzyme will 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

imatinib. 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 imatinib. 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 imatinib. 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.

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 doxorubicin, paclitaxel, and several other anticancer drugs. A simple DNA test 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.

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.

A 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. An understanding the molecular basis of clinical sensitivity or resistance to antiEGFR agents might identify patients who are likely to benefit from treatment with these MAbs. One study found that patients that were responsive to anti-EGFR antibody treatment had an increased number of copies of the EGFR gene when compared with a patient that did not respond to treatment (Moroni et al. 2005). The results suggest that MAbs are likely to be more effective against gene targets in cancer that are amplified rather than those affected by point mutations. Therefore, assessment of EGFR copy number might identify patients with metastatic CRC who are likely to respond to MAbs against EGFR. Those not likely to respond would be spare the expense and potential adverse effects of this treatment.

Examples of Personalized Management of Cancer

Personalized Management of 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 ER positive and negative cancers. Breast cancer can be typed into the following categories with distinct differences in prognosis and response to therapy:

- ER positive or ER negative: 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.

Genetic 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 EGFR-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 to 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 CPM, methotrexate, and fluorouracil (Pritchard et al. 2006).

Various methods have been used to analyze the HER2 status of a tumor:

- 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.

Pharmacogenetics of Breast Cancer

Polymorphisms in tamoxifen metabolizing genes affect the plasma concentration of tamoxifen metabolites. In a study, 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.

Molecular Diagnostics in Breast Cancer

Early detection of metastases. Detection of CTCs using by immunomagnetics before initiation of first-line therapy in patients with metastatic breast cancer is highly predictive of progression-free survival and overall survival. This technology can aid in appropriate patient stratification and design of tailored treatments.

Fiber array scanning technology (FAST). 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.

Realtime qualitative PCR (realtime-qPCR) assays. These have been used to risk-stratify breast cancers based on biological 'intrinsic' subtypes and proliferation (Perrard 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 EGFR 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 the 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 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, and are 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.

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).

Racial Factors in the Management of Breast Cancer

Gene expression analysis has identified several breast cancer subtypes, including basal-like, human EGFR-2 positive/ER 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 to only 6.3 in whites. A higher prevalence of basal-like breast tumors and a lower prevalence of luminal 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 to be fatal than other types. 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.

Proteomics-Based Personalized Management of Breast Cancer

Nipple aspirate protein samples from a group of patients who had been diagnosed with unilateral primary invasive ductal breast carcinoma and had an apparently normal contralateral breast can be examined by 2D GE and mass spectrometry

(Mannello et al. 2009). The 2D GE analysis involves the use of 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 markers. 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 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.

Tests for Prognosis of Breast Cancer

Prognostic testing of all patients prior to treatment aligns with standard medical practice to distinguish patients by hormone status This information can also enable pharmaceutical companies to clearly define patient stratification that improves 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 ER and PR) 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 ER and PR) prior to treatment.
- 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. A tumor bank study demonstrated that HOXB13:IL17BR index is a strong independent prognostic factor for ER + node-negative patients irrespective of tamoxifen therapy (Ma et al. 2006). As a result of this study, these two biomarkers serve as the foundation of the AviaraDx Breast Cancer Profiling Technology.

The activity of the 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. Analysis of over 2,000 breast cancer patients has demonstrated that DACH1 correlates with tumor size, stage and metastasis, with its expression greatly reduced in metastatic breast cancer cells, but increased nuclear DACH1 expression predicts improved patient survival (Wu et al. 2006). The average survival was almost 40 months longer in women in whom their breast cancer continued to express DACH1. DACH1 gene reverts the cancerous phenotype, thus turning the cell back to a premalignant state, and it could be used as a prognostic marker for breast cancer. Other cell fate-determining genes are being examined in an attempt to identify new therapeutics for breast cancer and metastasis.

Researchers at Fox Chase Cancer Center (Philadelphia, PA) have identified an important gene, CEACAM6 (carcinoembryonic antigen-related cell adhesion molecule 6), which is involved in the spread of breast cancer that has developed resistance to long-term estrogen deprivation. The gene may prove to be a useful marker 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 were 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 showed 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 be an important biomarker for metastasis and a possible target for novel treatments for patients with metastatic breast cancer.

ER-negative basal breast cancer is a heterogeneous disease with at least 4 main subtypes. It has been shown that the 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 ER gene in the constellation increases the confidence that this information complements routinely assayed ER 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.

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, so 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 phosphatase and tensin homolog (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 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 TP 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 carcinogenesis 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 coadministration 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.

Predicting Response to Chemotherapy in Breast Cancer

Some of the methods used to predict response to therapy are:

Predicting response to trastuzumab treatment. (SPOT-Light®) HER2 CISH Kit (Life Technologies), which received premarket approval by the FDA in 2008, is based on a technology called chromogenic in situ hybridization (CISH). The test uses a DNA probe for the HER2 gene and predicts whether a breast cancer patient is a candidate for trastuzumab treatment. Current medical practice requires that all patients who are considered for trastuzumab treatment be tested for HER2 amplification

or overexpression. CISH test 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.

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 (Dose Schwarz et al. 2005). 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 was used to show that low tau expression renders microtubules are more vulnerable to paclitaxel and makes breast cancer cells hypersensitive to this drug (Rouzier et al. 2005). 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-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 carcinogenic 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 estrogen-receptor-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 PR 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 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).

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 new 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 was derived from tumor characteristics present after women with stage 2 and 3 breast cancer underwent 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 ERs. Women with a PEPI score of zero had almost no 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 return 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 12–18 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.

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 ER status, PR 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.

Prediction of Resistance to Therapy in Breast Cancer

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 doxorubicin used for the treatment of breast cancer. In a retrospective study of breast cancer patients who were treated with doxorubicin, archival tumor specimens were analyzed and the relationship of GRP78 expression level to "time to recurrence" (TTR), used as a surrogate marker 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 TTR. 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 RT in Breast Cancer

RT 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 RT. 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 RT (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 RT could be warned in advance or alternative treatments can be sought. Further work needs to be done 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 RT), 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 (Nuyten et al. 2006).

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 estrogen-receptor-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 (Paik et al. 2004).

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 ER 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 ER 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 (Buyse et al. 2006). 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 were then compared to those generated using the algorithms based on the custom microarray and showed an extremely high correlation of prognosis prediction between the original data and those generated using the custom mini-array (Glas et al. 2006). Therefore, the array is an excellent tool to predict outcome of disease in breast cancer patients.

TargetPrint® (Agendia). This FDA approved test enables quantitative determination of gene expression levels of the ER, PR 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 January 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. In 2008, the FDA approved this test in the US.

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.

Researchers at the University of Michigan Comprehensive Cancer Center (Ann Arbor, MI) are leading a new study 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. The TAILORx study 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 will enroll more than 10,000 women from 900 sites in the US and Canada. Women recently diagnosed with estrogen-receptor 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 University of Cincinnati in Ohio 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.

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, estrogen-receptor 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. During a phase II clinical trial in 2008, a team of researchers at Washington University School of Medicine (St. Louis, MO) was able to classify estrogen-receptor positive tumors into low-, medium-, and high-risk groups depending on the genetic signature in the tumors a month after patients started treatment. Rather than just looking for the specific gene signature in tumors before treatment, the researchers also tested expression of 50 genes after treatment with letrozole (Novartis' Femara), a drug that blocks estrogen production. The team identified a group of about 10 to 15% of estrogen-receptor

positive tumors that 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 new knowledge may eventually change the way that physicians design estrogen-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 has 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.

Future Development of Gene Expression Microarrays for Breast Cancer

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 (Miller and Liu 2007):

- 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.

Personalized Management of 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 models for the molecular characterization of pathway-targeted therapy. Response to a particular anticancer drug can be related to the signaling pathway involved. Effect of rapamycin on cell proliferation, tumor growth, and the accumulation of peritoneal

ascites were investigated in this model using both in vitro and in vivo approaches (Xing and Orsulic 2005). 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, 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 results 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 pathwaytargeted 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.

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 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 6 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 Precision Therapeutics Inc. (PTI) developers of the ChemoFX assay. PTI exclusively licensed the Yale apoptosis assay from Yale.

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. A side population (SP) cells has been identified and characterized from two distinct genetically engineered mouse ovarian cancer cell lines (Szotek et al. 2006). Differential efflux of a DNA-binding dye from these cell lines defined the human breast cancer-resistance protein 1-expressing, verapamil-sensitive SP of candidate cancer stem cells. In vivo, mouse SP cells formed

measurable tumors sooner than non-SP (NSP) cells when equal numbers were injected into the dorsal fat pad of nude mice. The presence of Mullerian Inhibiting Substance (MIS) signaling pathway transduction molecules in both SP and NSP mouse cells led us to investigate the efficacy of MIS against these populations in comparison with traditional chemotherapies. MIS inhibited the proliferation of both SP and NSP cells, whereas the lipophilic chemotherapeutic agent doxorubicin more significantly inhibited the NSP cells. Finally, breast cancer-resistance protein 1-expressing verapamil-sensitive SPs 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.

Scientists at the NIH have developed a gene expression profile that predicts ovarian cancer patient response to chemotherapy. One gene signature can predict whether a patient will initially respond to standard platinum-paclitaxel chemotherapy, but will relapse within 6 months of completing treatment. A second gene signature identifies patients who will show no response to therapy. This method may enable clinicians to identify patients who may be candidates for additional and/or novel chemotherapy drugs, and effectively choose appropriate cancer treatment. A unique feature of this signature is its derivation from pure, microdissected isolates of ovarian tumor cells, rather than undissected tissue. An advantage of this approach is that the resulting gene list is specific to the cell type which causes the disease.

Two tumor biomarkers, CA125 and one recently approved by FDA called HE4, are used to track whether chemotherapy is working or cancer is recurring. A one-time CA125 test can not 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.

OvaSure (LabCorp) measures concentrations of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 by using a multiplex, bead-based, immunoassay system. OvaSure is a screening test for women at high risk of ovarian cancer that was developed by Yale University under a law that allows a single laboratory to offer testing without FDA review. Used on blood samples stored from cancer patients and healthy women, the test correctly identified cancer a sensitivity of 95.3% and a specificity of 99.4% (Visintin et al. 2008). However, this does not prove that OvaSure can detect when cancer is forming. Efforts to validate OvaSure are ongoing.

Human ovarian cancer stem cells (OCSCs) 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 cancer cells 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 OCSCs can aid in the development of better therapeutic approaches for ovarian cancer patients.

Personalized Management of Hematological Malignancies

Considerable work has been done on molecular cytogenetics of hematological malignancies and a number of diagnostics and therapies are available or under development. Myeloproliferative disorders include several pathologies sharing the common feature of being clonal hematopoietic stem cell diseases. The molecular basis of 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.

Ipsogen has worldwide exclusive intellectual property rights to a test based on mutations in the JAK2 gene. It has signed of an agreement with Laboratory Corporation of America, which will offer a JAK2 molecular diagnostic assay in the USA.

Personalized Management of Acute Leukemias

Progress in the molecular classification of 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. An example is imatinib mesylate for the treatment of BCR-ABL-positive CML. This agent, which inhibits the BCR-ABL fusion protein and other constitutively active tyrosine kinases and which has induced transient remissions of BCR-ABL-positive ALL and 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 MRD after administration of mercaptopurine in the early course of childhood ALL, most likely through modulation of mercaptopurine dose intensity (Stanulla et al. 2005). 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 (Memphis, TN) 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.

Gemtuzumab ozogamicin, an approved MAb conjugated with a cytotoxic antitumor antibiotic calicheamicin, is used to treat patients with acute myelogenous leukemia (AML). The antibody portion of this drug binds specifically to the CD33 antigen, a sialic acid-dependent adhesion protein found on the surface of leukemic blasts and immature normal cells of myelomonocytic lineage, but not on normal hematopoietic stem cells. Binding results in the formation of a complex that is internalized. Upon internalization, the calicheamicin derivative is released inside the lysosomes of the myeloid cell. The released calicheamicin derivative binds to DNA in the minor groove resulting in DNA double strand breaks and cell death. Because of its targeted delivery to specific cells and selective action, it can be considered a personalized medicine.

Two molecular tests for acute myeloid leukemia (AML) are relevant to personalized management: FLT3 Mutation Analysis and WT1 RQ-PCR (Genzyme Diagnostics). 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.

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).

Personalized Management of Chronic Lymphocytic Leukemia

B-cell chronic lymphocytic leukemia (CLL) is the second most common leukemia, 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, or special white blood cells, in the bone marrow. These cells can overwhelm the bone marrow and invade the blood stream, eventually spreading to the spleen, liver and other solid organs. The elimination of CLL to an extremely low level may improve the overall survival and treatment-free survival. According to a study, 84% of patients who had no detectable CLL cells after receiving alemtuzumab had survived for at least 5 years; 20% of the same patients had previously failed to respond or had relapsed after receiving other chemotherapy for their disease (Moreton et al. 2005). CLL patients who relapse from or are refractory to chemotherapy have the poorest prognosis with a median survival of 10 months. A companion test to detect MRD in patients with B-cell CLL complement the treatment with alemtuzumab. This is an example of combining diagnostics with therapy to improve the treatment.

Personalized Management of Multiple Myeloma (MM)

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 USA 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 MM is only 32% and durable responses are rare. MM 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 has been observed that different abnormalities vary from one patient to the other.

Pharmacogenomic studies in MM are helping to set the stage for individualized therapy. Although relatively few in number, these studies are already providing new therapeutic targets and avenues for drug discoveries as well as contributing to novel prognostic markers in MM. 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 MM.

A gene profiling technique may eventually enable oncologists to prescribe "personalized" treatments for individual patients with MM. It involves use of microarray technology to determine which of the estimated 12,000 human genes are "turned on" or "turned off" in MM cells and segregated MM into different groups according to gene profiles. The new classification system is based on

similarities of myeloma to different stages of normal plasma cell development and is linked to historically important clinical parameters used in prognosis. The goal is to use the gene "profiles" to classify cases of MM according to how patients respond to different treatments. By classifying individual patients according to their gene profiles, physicians will be able to practice "personalized medicine" by choosing experimental treatments for patients whose profiles suggest that they will not live long on conventional therapy. The variability in myeloma survival is considerable, with some patients succumbing within months while others can live for a decade. Currently only 20% of this variability can be explained. Although the median survival rate for MM in the US is 2.5–3 years, the personalized approach described raised the median survival rate to 6–7 years.

Four distinct genetic subtypes of MM have been identified that have different prognoses and might be treated most effectively with drugs specifically targeted to those subtypes (Carrasco et al. 2006). For further analysis many DNA alterations in the myeloma genome, the authors created an algorithm based on a computational method, non-negative matrix factorization, designed to recognize individuals by facial features. The algorithm was used to group the results in a way that yielded distinctive genomic features from the CGH data. Four distinct myeloma subtypes based on genetic patterns emerged: two of them corresponded to the non-hyperdiploid and hyperdiploid types, but the latter was found to contain two further subdivisions, called k1 and k2 When these subgroups were checked against the records of the patients from whom the samples were taken, it showed that those with the k1 pattern had 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 treatments 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 works, and they have identified nine new genetic mutations in cancer cells that should increase a patient's chance of responding to the agent, and may help physicians tailor treatment to patients. Bortezomib seems to work in about one-third of patients who use it, but up to now it is difficult 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 Nuclear factor-kappaB (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.

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 conducting clinical trials of the new targeted therapy to enroll patients 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 for them.

Personalized Vaccine for Follicular Lymphoma

Follicular lymphoma is considered incurable, although CPM, 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.

Personalized Management of Myelodysplasia

In MDS, cytogenetic analyses are mandatory for risk stratification and for monitoring response to drug treatment. Low-dose demethylating agents such as 5-aza-2¢-deoxycytidine (decitabine) and 5-azacytidine (azacitidine) have been explored for the treatment of MDS aiming to revert a methylator phenotype. Cytogenetic subgroups as predictors of response to low-dose decitabine and demethylating agents in MDS. 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.

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. 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.

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 in the near future to evaluate an individual's hereditary risk for developing melanoma. Several pharmacogenomic-based therapies are in early stages of development for melanoma.

Personalized Management of Gastrointestinal Cancer

Personalized Management of Esophageal Cancer

Esophageal cancer is a highly aggressive malignancy. Almost half of the 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 anti-metabolite chemotherapy agent (5FU), an alkylating agent (cisplatin) and radiation treatment. Researchers from the MD Anderson Cancer Center (Houston, TX) have reported six different gene variants that can predict an improved outcome in patients treated with two different chemotherapy drugs and/or with radiation therapy. They have conducted a study to evaluate esophageal cancer treatment with a pharmacogenetic paradigm and to apply pharmacogenetic analysis to multiple genes in each drug action pathway as a means of developing a more accurate and consistent risk prediction model (Wu et al. 2005). The preliminary finding on patients with resectable adenocarcinoma or squamous cell carcinoma of the esophagus who had been treated with chemoradiation followed by esophagectomy show that methylenetetrahydrofolate reductase (MTHFR) polymorphisms can modify 5-FU 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. The ongoing esophageal cancer research 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 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 were those who had gene variants that were less effective at neutralizing the killing power of the cancer treatments. Conversely, patients whose genes efficiently counteracted chemotherapy and radiation treatment had shorter survival times overall. Another finding of the study was an additive effect between these genes and others that conferred smaller advantages. The higher the number of beneficial variants the patient had, the longer survival was. If successful, such pathway-based analyses can be conducted for the wide variety of cancers that are treated with 5-FU, cisplatin and radiation, as well as other drug treatments.

Personalized Management of CRC

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 nearly 150,000 Americans diagnosed with the disease in 2008. The cause of CRC is multifactorial, involving hereditary susceptibility, environmental factors, and somatic genetic changes during tumor progression. 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 5–10% 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 non-steroidal antiinflammatory drugs (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 PDs and PKs. 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/RT 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 marker 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.

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 the drug. Functional analysis of three genes identified in the microarray study (prostate-derived factor, calretinin, and spermidine/spermine *N*1-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 is a recombinant, human IgG2 kappa monoclonal antibody 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 (DxS Ltd.), 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 oncogene. 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 marks the first time that the European Commission has licensed a bowel cancer treatment with the stipulation that a predictive test should be carried out.

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 CRC 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. Interest is focused on DNA methylation, an epigenetic mechanism that is involved in everything from imprinting to X-chromosome inactivation. 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 CRC that predicts cancer recurrence and may be considered a prognosis score (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 retrovirus-associated DNA sequences (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 ten 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 growth, node, metastasis, or cancer classification system based on Tumor, Nodes and Metastases (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 colon cancer and improve survival. Clinical trials are planned to test usefulness if this approach.

Identification of genetic factors underlying drug response in CRC still remains a promising areas for improving management of CRC patients. Genetic variations identified in genes encoding TS, DPD, 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). However, additional investigation is needed to validate fully the clinical relevance of individual genetic differences.

Personalized Management of Lung Cancer

Determination of Outcome of EGFR Tyrosine Kinase Inhibitor Treatment

The tyrosine kinase inhibitor gefitinib, which targets the EGFR, is approved for late cases of 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 make excess 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. A study from the Massachusetts General Hospital/Dana Farber Cancer Institute (Boston, MA) indicates that response of lung cancer patients to gefitinib is determined

by a certain mutation in the EGFR gene (Lynch et al. 2004). Eight of nine patients who responded to gefitinib had mutation-containing tumors; seven patients not helped by gefitinib did not. 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 epidermal growth factor 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 were normal receptors. The mutations are more common in women, people who had never or not recently smoked, and people who had a subtype called bronchoalveolar cancer. Similar results were obtained in another study where receptor tyrosine kinase genes were sequenced in NSCLC and matched normal tissue (Paez et al. 2004). EGFR mutations were found in additional 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 marker for response to EGFR inhibitors, stable disease, time to progression, and survival in NSCLC (Hirsch and Witta 2005). However, EGFR mutation is a better predictor of clinical outcome in gefitinib-treated patients than the EGFR gene copy number (Endo et al. 2006). 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 erlotinib and 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.

Another drug targeting the EGFR receptor is erlotinib. A randomized, placebo-controlled, double-blind trial was conducted to determine whether erlotinib prolongs survival in NSCLC after the failure of first-line or second-line chemotherapy (Shepherd et al. 2005). Presence or absence of EGFR mutation was not taken into consideration. The results show that erlotinib can prolong survival in patients with NSCLC after first-line or second-line chemotherapy. A clinical trial has compared responsiveness to erlotinib with a placebo for NSLC using tumor-biopsy samples from participants in this trial to evaluate EGFR expression immunohistochemically (Tsao et al. 2005). The results indicate that among patients with NSCLC who receive erlotinib, the presence of an EGFR mutation may increase responsiveness to the agent, but it is not indicative of a survival benefit.

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. A study with gefitinib and cetuximab (Erbitux), a MAb drug for colon cancer, has shown that although both drugs killed cells containing a normal but

overactive EGFR molecule, only gefitinib killed lung cancer cells containing a mutated EGFR molecule whereas cetuximab had little effect on the mutant signal, evidently because it strikes at a different part of the EGFR molecule (Mukohara et al. 2005). Thus those with EGFR mutations will benefit from gefitinib or erlotinib, while another group, without EGFR mutations, will 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 performed differently against the mutant EGFR cells. These studies 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 ECD.

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 (DxS Ltd.) 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 over 72% of the paired samples. The DxS 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 (DxS), available in Europe, detects mutations that correlate with responsiveness to EGFR tyrosine kinase inhibitors. This test may be used to help physicians choose lung cancer patients who are most likely to respond to treatment with EGFR tyrosine kinase inhibitors.

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 CTCs 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 CTCs from the blood of patients with lung cancer offers the possibility of monitoring changes in epithelial tumor genotypes during the course of treatment.

Testing for Response to Chemotherapy in Lung Cancer

To gain insight into clinical response to PBC in NSCLC, matched tumor and non-tumor 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 had a statistically significant better response to chemotherapy compared with those with the other haplotypes combined (Sohn et al. 2006). These findings suggest that the MDR1 polymorphisms can be used for predicting treatment response to etoposide-cisplatin chemotherapy in SCLC patients.

Testing for Prognosis of NSCLC

An automated quantitative determination of the RRM1 protein, the regulatory subunit of ribonucleotide reductase involved in the response of NSLC to treatment, has been developed in routinely processed histologic specimens (Zheng et al. 2007). The expression of RRM1 and two other proteins that are relevant to NSCLC – the excision repair cross-complementation group 1 (ERCC1) protein and the phosphatase and tensin homologue (PTEN) – were measured. The results were compared with the clinical outcomes in patients with early-stage NSCLC who had received only surgical treatment. The survival advantage was limited to the 30% of patients with tumors that had a high expression of both RRM1 and ERCC1 indicating that these are determinants of survival after surgical treatment of early-stage, NSCLC.

Testing for Recurrence of Lung Cancer

The lung metagene model. This model is based on gene expression profiles to predict the risk of recurrence in patients with early-stage NSCLC (Potti et al. 2006a). A sample of the tumor is taken as it is removed during surgery. Its mRNA is extracted, labeled with fluorescent tags and placed on a gene chip where it binds to its complementary DNA sequence. When scanned with special light, the fluorescent RNA emits a luminescence that demonstrates how much RNA is present on the chip and thus, which genes are most active in a given tumor. The physicians then use a rigorous statistical analysis to assess the relative risk of large grouping of genes, called metagenes, which have similar characteristics. The test generates a risk "number" for each patient. If their risk exceeds 50%, the patient is advised to get chemotherapy. The model predicted recurrence for individual patients significantly better than did clinical prognostic factors and was consistent across all early stages of NSCLC. It identified a subgroup of patients who were at high risk for recurrence and who might be best treated by adjuvant chemotherapy. The lung metagene model thus provides a potential mechanism to refine the estimation of a patient's risk of disease recurrence and, in principle, to alter decisions regarding the use of adjuvant chemotherapy in early-stage NSCLC. It is the first-ever genomic test to predict which patients with early-stage NSCLC will need chemotherapy to live and which patients can avoid the toxic regimen of drugs. This is an example of personalized management of lung cancer.

Five-gene signature for predicting survival. 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.

Role of microRNAs. miRNAs have been shown to control the expression of cognate target genes and predict relapse in surgically resected NSCLC patients (Rosell et al. 2006). 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 both in 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.

Personlized 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. A test can predict which prostate cancer patients will benefit from an experimental therapy that blocks a cell signaling pathway responsible for driving the growth of the cancer (Thomas et al. 2004). It showed, for the first time, in tissues from men with prostate cancer 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 mammalian target of rapamycin (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. The discovery may allow oncologists to 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. About 230,000 men will be diagnosed with prostate cancer in the USA in 2009. Of those, about 25–30% are 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.

Benefit 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 carcinogenesis, 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.

Personalized Management of Brain Cancer

Glioblastoma multiforme (GBM), the most malignant and most frequent brain tumor is currently incurable with a median survival of less than 2 years after diagnosis and treatment. Worldwide approximately 175,000 cases occur annually of which 17,000 are diagnosed in the USA. 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.

Genetics and Genomics of Brain Cancer

Genetic alterations in GBM have been studied extensively using molecular diagnostic technologies (Jain 2009k). 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 (Liang et al. 2005). DNA biochips have been used to identify tumors with the best prognosis, whose chromosome 1 has undergone a

specific deletion (Idbaih et al. 2005). 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. Results were obtained by studying the specific genetic alterations of a subgroup of more chemosensitive gliomas. These findings were recorded using high-density array-comparative genomic hybridization (CGH) analysis. CGH chips are made by using targets from genome fragments of about 150,000 base pairs. With some 3,500 targets, these chips afford an overview of the whole genome. This technique can establish high-resolution maps revealing genome anomalies (amplifications, deletions). Screening for these deletions can be incorporated into standard diagnostic tests for GBM. In 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 glioblastoma (malignant brain tumor) 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. One study reports that STAT3 plays 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 EGFR 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.

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 upregulate 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 glioblastoma. One of the next steps is to determine whether these patients may respond better to drugs targeting the NF-κB network.

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. The techniques used to obtain this molecular information, as well as the advantages and disadvantages of the different techniques have been discussed elsewhere (Jeuken et al. 2006). 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 malignant glioma were examined by diffusion MRI before treatment and 3 weeks after treatment; the images were coregistered, and differences in tumor-water diffusion values were calculated as functional diffusion maps (fDM), which were correlated with the radiographic response, time-to-progression, and overall survival (Moffat et al. 2005). Changes in fDM at 3 weeks were closely associated with the radiographic response at 10 weeks. The percentage of the tumor undergoing a significant change in the diffusion of water was different in patients with progressive disease as compared to those with stable disease. fDM provide an early biomarker for response, time-to-progression, and overall survival in patients with malignant glioma. 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 should 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 et al. 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 potential clinical usefulness for predicting the efficacy of anticancer agents. In one proteomic study, surgical samples of human gliomas were analyzed with two-dimensional gel electrophoresis (2D GE) and mass spectrometry and in vitro chemosensitivities to various anticancer agents (e.g., CPM, nimustine, cisplatin, cytosine arabinoside, mitomycin C, doxorubicin, etoposide, vincristine, paclitaxel) were measured by flow cytometric detection of apoptosis (Iwadate et al. 2005). Proteins that significantly affected the in vitro chemosensitivity to each category of anticancer agents were identified. Many of the proteins that correlated with chemoresistance were categorized into the signal transduction proteins including the G-proteins. This study showed that the proteome analysis using 2D GE could provide a list of proteins that may be the potential predictive markers 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.

Personalized Chemotherapy of Brain Tumors

Although approximately 26% of patients treated with temozolomide survive more than 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.

MGMT gene promoter methylation testing. A clinical trial conducted at the University Hospital of Lausanne in Switzerland found that activity status of a single gene could predict response to therapy (Hegi et al. 2005). The O6-methylguanine-DNA-methyltransferase (MGMT) promoter was methylated in 45% of 206 assessable cases. Irrespective of treatment, MGMT promoter methylation was an independent favorable prognostic factor. Among patients whose tumor contained a methylated MGMT promoter, a survival benefit was observed in patients treated with temozolomide and RT; their median survival was 21.7 months as compared with 15.3 months among those who were assigned to only RT. In the absence of methylation of the MGMT promoter, there was a smaller and statistically insignificant difference in survival between the treatment groups. Testing for the methylation status of the MGMT gene by PCR could lead to the use of temozolomide as first-line therapy in those identified as responder patients. Further analysis of the genetic pattern of the tumor after biopsy might provide new drug targets for the disease. Stratification according to MGMT promoter methylation status may be considered in future trials in which temozolomide or other alkylating agents are used.

In March 2009, OncoMethylome Sciences started MGMT gene promoter methylation testing in a in a phase II clinical trial (CORE trial) for cilengitide in newly diagnosed GBM patients. In addition, testing is also being performed in a phase III clinical trial (CENTRIC trial) in newly diagnosed glioblastoma that has been running since 2008. Patient selection for those trials is based on the MGMT gene promoter methylation status of their tumor tissue.

Molecular determinants of response to EGFR inhibitors. EGFR is frequently amplified, overexpressed, or mutated in glioblastomas, but only 10–20% of patients have a response to EGFR kinase inhibitors. In patients with recurrent malignant glioma, coexpression of EGFRvIII and PTEN by glioblastoma cells is associated with responsiveness to EGFR kinase inhibitors (Mellinghoff et al. 2005).

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 PKs and PDs 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 GBM patient treatments. 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.

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.

Personalized Therapy of Oligodendroglial Tumors

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. Scientists at the University Medical Center (Nijmegen, The Netherlands) are developing novel predictive diagnostic tools for personalizing the treatment of OTs by aiming to (i) define a molecular profile capable of identifying all Procarbazine-Lomustine-Vincristine (PCV)-chemosensitive gliomas and (ii) identify genes/signaling pathways involved in PCV chemosensitivity.

Anaplastic oligodendroglioma (AO) and anaplastic oligoastrocytoma (AOA) are treated with surgery and RT at diagnosis, but they also respond to procarbazine, lomustine, and vincristine (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 (Intergroup Radiation Therapy Oncology Group Trial 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 RT 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, pinpointing the optimum dosage to treat neuroblastoma can be tricky. Researchers at St. Jude Children's Research Hospital (Memphis, TN) have shown that finding the optimal dosage of the drug topotecan improves the efficacy of treatment of children with neuroblastoma. From the results of a number of earlier studies, they found that giving a low topotecan dosage on an extended schedule was the best way to destroy tumors. More recently they found that if close monitoring and fine- tuning topotecan drug levels for each child by a technique called PK-based (PK-based) dosing improves the response to treatment. PK-based dosing reduces variability in the amount of topotecan in the body, leading to improvements in response and ultimately improving 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 (Jarnjak-Jankovic et al. 2005).

Personalized Management of Germ Cell Brain Tumors

A phase II study was carried to determine response to chemotherapy and survival after response-based 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 + 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.

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

The 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 PK and metabolic properties; and (2) the development of validated PD 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 limitation of genotyping for MTHFR, which plays a central role in the action of 5-FU, an inhibitor of TS, by converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two polymorphisms in the MTHFR gene (677C > T and 1298 A > C) have been considered as genomic predictors of clinical response to fluoropyrimidine-based chemotherapy (in combination with irinotecan or oxaliplatin). The results of a study on patients with metastatic CRC and undergoing 5-FU-containing chemotherapy as a first line treatment suggest that the MTHFR genotype cannot be considered as an independent factor of outcome (Marcuello et al. 2006).

The 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 (GBM), lung (squamous carcinoma), and ovarian (serous cystadenocarcinoma). Together, these cancers account for more than 258,480 cancer cases each year in the USA.

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.

Role of the International Cancer Genome Consortium

In April 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.

Using Computer and Imaging Technologies to Personalize Cancer Treatment

In 2008, the Cancer Institute of New Jersey and IBM started collaboration to develop more accurate diagnostic tools aimed at improving cancer treatments and outcomes. They will use 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, funded by a $2.5 million grant from the NIH, is 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 P6 570 series class systems to the Center, which uses grid technology that allows collaborators from around the country access the Center's database and software.

Integrated Genome-Wide Analysis of Cancer for Personalized Therapy

An integrated genome-wide analysis of CNV in breast and CRCs 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 CRCs. Since several breast cancer samples tested contained changes to DNA topological pathways, some of these tumors may be candidates for topoisomerase-targeted therapies.

Summary

Cancer is the area with the greatest need for personalized therapy. Considerable advances have already taken place in molecular diagnostics of cancer, understanding of the molecular mechanisms, and combination of diagnostics with therapeutics. A new molecular classification of cancer is relevant to personalized management. Part of the progress is due to integration of new technologies relevant to cancer for personalizing management. Functional diffusion MRI and FDG-PET are important imaging technologies for development of personalized management of cancer. Cancer biomarkers are important for developing diagnostics as well as therapeutics of cancer. Among various technologies nanobiotechnology and proteomics are making major contributions to the development of personalized therapy of cancer. Pharmacogenomic approaches can make cancer chemotherapy more effective and spare the patients unnecessary toxicity of ineffective treatments. 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. Physical modalities of treatment of cancer such as radiation therapy can also be personalized. Finally examples of personalized management of cancers involving different organs are presented.

Chapter 11
Personalized Management of Neurological Disorders

Introduction

Personalized neurology requires the integration of several neuroscientific and clinical aspects of neuropharmacology (Jain 2005c). Drug discovery for neurological disorders should take into consideration targeting a specific type in the broad clinical category of a neurological disease in the conventional clinical diagnosis. Drug delivery to the central nervous system (CNS) is an important factor in personalizing treatment of neurological disorders. Personalized management of some important neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), epilepsy, migraine, and multiple sclerosis (MS) will be considered in this chapter.

Personalized Drug Development for Neurological Disorders

Personalized Drug Discovery

CNS drug candidates fail approval in over 90% of the cases owing to problems in the delivery to the site of action in the brain, lack of efficacy, and unacceptable side effects. New drugs are badly needed for CNS disorders. The greatest activity is in the use of biomarkers as potential drug targets, but those for disease mechanism, efficacy, and toxicological effects are under investigation. Many of the biomarkers can later be developed as new diagnostic agents to guide personalized molecular therapy (Frost 2008).

Molecular Imaging and CNS Drug Development

In vivo imaging offers a pathway to reduce the risk of failure of drug molecules at each stage of development, but more research and development is needed to fully

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realize this potential. However, 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 given below.

Multiple sclerosis (MS). ^{11}C-PK11195 can pick up inflammatory changes in both optic nerves in MS patients, which do not show up on ordinary MRI. It fulfils the need for a marker as a guide to interferon therapy for these patients.

Parkinson's disease (PD). ^{11}C-PK11195 PET can be used to follow the progression of inflammation in PD 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's 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 beta-amyloid 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.

Personalized Management of AD

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. Pharmacogenomic aspects were described briefly in Chapter 4.

The diagnosis of AD is currently based on clinical and neuropsychological examination. There is currently no biomarker of AD for early detection. MRI and computer tomography (CT) scan images of hippocampus shrinkage and, later on, global brain shrinkage are used to help diagnose advanced disease. To date there is no definitive blood test available that can discriminate dementia patients from healthy individuals. A combination of characteristic plaque markers tau and amyloid β (Aβ) may constitute a specific and sensitive cerebrospinal fluid 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 the 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 (Jain 2009o). Numerous neuroprotective therapies are under investigation but the only one currently marketed is memantine – a non-competitive *N*-methyl-D-aspartate antagonist. 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 the 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.

Functional genomics, proteomics, pharmacogenomics, high-throughput methods, combinatorial chemistry, and modern bioinformatics will greatly contribute to accelerate drug development for AD. 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 produced conflicting results. A multifactorial therapy combining three different drugs yielded positive results during the 6–12 months in approximately 60% of the patients (Cacabelos 2002). With this therapeutic strategy, APOE-4/4 carriers were the worst responders, and patients with the APOE-3/4 genotype were the best responders. 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.

Various isoforms of the nitric oxide (NO) producing NO synthase (NOS) are elevated in AD indicating a critical role for NO in the pathomechanism. The potential structural links 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 investigated in AD. 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 possible 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 finally the implementation of new tools towards a more personalized management of AD (Barba et al. 2008).

Personalized Management of PD

PD is characterized by progressive degradation of dopaminergic (DA) 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 the 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 the effect of drugs that work by neurochemical intervention.

Cytochrome P450 CYP2D6 enzyme, which metabolizes many drugs, is also involved in the metabolism of dopamine. Prevalence of CYP2D6 4 allele differs significantly between the PD patients and normal subjects.

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 the levodopa dose. However, 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.

Discovery of Subgroup-Selective Drug Targets in PD

Studies using global gene-expression profiles define the four major classes of DA and noradrenergic neurons in the brain. The molecular profiles obtained provide a basis for understanding the common and population-specific properties of catecholaminergic (CA) neurons and will facilitate the development of selective drugs. One of their goals 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. They also observed selective high expression of γ-synuclein in the neurons of the SN and in the 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.

Personalized Management of Epilepsy

Epilepsy is characterized by excessive neuronal activity (seizures) in the brain, typically causing muscle spasms, convulsions, and altered behavior. It is one of the most common neurological disorders and afflicts approximately 1–1.5% of the

population, i.e., approximately 50 million people affected world-wide. At least 2.5 million people in the US suffer from epileptic seizure disorders and 125,000 new cases are diagnosed every year. At least 20 different types of epilepsy have been identified. These patients can usually be divided into two major types: partial seizures (seizures that begin in a localized area of the brain)/epilepsy and generalized seizures/epilepsy. The mainstay of treatment is pharmacotherapy and the primary criterion for the selection of AED is the patient's seizure type.

Choice of the Right AED

Current treatment of epilepsy is imprecise. The mainstay of treatment for epilepsy is pharmacotherapy and 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. 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.

Pharmacogenetics of Epilepsy

Pharmacogenetic alterations can affect efficacy, tolerability, and safety of AEDs, including variation in genes encoding drug target (SCN1A), drug transport (ABCB1), drug metabolizing (CYP2C9, CYP2C19), and human leukocyte antigen (HLA) proteins. The current studies associating particular genes and their variants with seizure control or adverse events have inherent weaknesses and have not provided unifying conclusions. However, several observations, for example, that Asian patients with a particular HLA allele, HLA-B*1502, are at a higher risk for Stevens-Johnson syndrome when using carbamazepine, are helpful in improving our knowledge of how genetic variation affects the treatment of epilepsy (Löscher et al. 2009). A better understanding of the genetic influences on outcome of

epilepsy is a key to developing the much needed new therapeutic strategies for individual patients with epilepsy.

Pharmacogenomics of Epilepsy

One of the difficulties in managing epilepsy is that the cause is unknown with the exception of seizures because of known pathology such as brain tumors and head injury. Epilepsy is mostly a multifactorial disorder although familial forms occur and some epilepsy genes have been identified. Currently there are no genetic tests for epilepsy. SNP association analysis shows that malic enzyme 2 (ME2) gene predisposes to idiopathic generalized epilepsy (Greenberg et al. 2005). ME2 is a genome-coded mitochondrial enzyme that converts malate to pyruvate and is involved in neuronal synthesis of the neurotransmitter gamma-aminobutyric acid (GABA). Disruption of the synthesis of GABA predisposes to seizures, which are triggered when mutations at other genes are present. It is also becoming increasingly clear that genetic polymorphisms play an integral role in variability in both AED pharmacokinetics and pharmacodynamics. 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 antiepileptic therapy (Tang et al. 2004). The publication of the human genome and increasing sophisticated and powerful genetic tools offers new methods for screening drugs and predicting serious idiosyncratic side effects.

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 affects 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. Variants of two genes have been identified that are more likely to be found in patients who require higher dosages of AEDs carbamazepine and phenytoin (Tate et al. 2005). One variant of the gene which encodes CYP2C9 shows a significant association with the maximum dose of phenytoin taken by patients with epilepsy. Moreover, a variant of a second gene, called SCN1A, with activity in the brain, is found significantly more often in patients on the highest doses of both carbamazepine and phenytoin. SCN1A has been implicated in many inherited forms of epilepsy and is the drug target for phenytoin. Detection of these gene variants might determine, 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. Such a trial might also enable physicians to identify patients who might safely take a smaller dose, thereby minimizing their risk for adverse side effects.

Drug Resistance in Epilepsy

Another problem with current therapy 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. Because this form is resistant to multiple AEDs, the mode of resistance must be nonspecific, involving drug-efflux transporters such as ATP-binding cassette sub-family B member 1 (ABCB1, also known as MDR1 and P-glycoprotein 170). A genotyping study has shown that patients with drug-responsive epilepsy, as compared to patients with drug-resistant epilepsy, were more likely (28% vs. 16%) to have the CC genotype at ABCB1 3435 than the TT genotype (Siddiqui et al. 2003). The polymorphism fell within an extensive block of linkage disequilibrium spanning much of the gene, implying that the polymorphism may not itself be causal but rather may be linked with the causal variant. The results of this study indicate that a genetic factor is associated with resistance to AEDs and suggest new avenues for early molecular prediction of drug resistance. Since 2003, several other association genetics studies have sought to confirm this result, but did not support a major role for this polymorphism. 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.

Cellular mechanisms underlying drug resistance have been studied by comparing resected hippocampal tissue from two groups of patients with temporal lobe epilepsy (TLE); the first displaying a clinical response to the anticonvulsant carbamazepine and a second group with therapy-resistant seizures (Remy et al. 2003). It was shown that the mechanism of action of carbamazepine, use-dependent block of voltage-dependent Na^+ channels, is completely lost in carbamazepine-resistant patients. Likewise, seizure activity elicited in human hippocampal slices is insensitive to carbamazepine. In marked contrast, carbamazepine induced use-dependent block of Na^+ channels and blocked seizure activity in vitro in patients clinically responsive to this drug. These data suggest that the 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.

A study of the properties of transmitter receptors of tissues removed during surgical treatment of drug-resistant TLE show use-dependent rundown of neocortical $GABA_A$-receptor (Ragozzino et al. 2005). 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.

Future Prospects for Epilepsy

For the future, it is expected that several gene mutations will be identified in epilepsy using DNA biochips, e.g., those in ion channel genes. Future drugs may be designed specifically according to the electrophysiological dysfunction as personalized medicines for 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 new potential 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. In order to begin to have clinical impact, the following recommendations have been made (Ferraro et al. 2006):

- Standards specific to the conduct of future AED studies must be established, particularly accurate epilepsy classification, appropriate AED selection, and clear and objective assessment outcome measures.
- General standards for analysis and interpretation of genetic association data must be better codified and applied consistently across studies.
- Extensive clinical research networks must be formulated and large numbers of well characterized patients must be recruited.

- Further development of these critical factors will optimize chances for overcoming current challenges posed by AED pharmacogenetic research and ultimately allow the realization of improved, more rational therapeutic strategies.

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. 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. Genetic profiling of predisposition to migraine should facilitate the development of more effective diagnostic and therapeutic applications. 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). Pharmacogenomics will most likely provide a stronger scientific basis for optimizing drug therapy on the basis of each patient's genetic constitution (Tfelt-Hansen and Brøsen 2008).

Personalized Treatment of MS

MS is considered to be an autoimmune disease associated with abnormalities in immune regulation. Although the 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, they have common adverse effects and impose a high cost on health systems. The wide heterogeneity of MS and the 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. An MS assay, gMS™ (Glycominds), enables staging of the predicted disease activity and identification of the most appropriate treatment strategy in patients presenting with a first demyelinating events.
3. 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.
4. The current focus in the treatment of MS 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 MS, is a neuroprotective agent. A statistically significant association has been detected between glatiramer acetate response and a single nucleotide polymorphism in a TCR-β variant in patients with MS (Grossman et al. 2007).
5. MRI has become established as a reliable, sensitive, and reproducible technique for studying the pathophysiology of MS and provides a means for optimizing treatment for individual patients.
6. Early, active MS 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. 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 (Keegan et al. 2005). 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.

MBP8298

MBP8298 (BioMS Medical) is a synthetic peptide that consists of 17 amino acids linked in a sequence identical to that of a portion of the human myelin basic protein (MBP). MBP8298 has been developed for the treatment of MS. The specificity of

the immune attack in MS at the molecular level is determined in each case by the HLA type of the individual patient, and HLA type is known to be one factor that contributes to susceptibility to MS. The MBP8298 synthetic peptide is a molecular replicate of the site of attack that is dominant in MS patients with HLA haplotypes DR-2 or DR-4. These HLA types are found in 65–75% of all MS patients.

The apparent mechanism of action of MBP8298 is the induction or restoration of immunological tolerance with respect to the ongoing immune attack at this molecular site. High doses of antigen delivered periodically by the intravenous route are expected to suppress immune responses to the administered substance. The potential benefit of MBP8298 for any individual patient is therefore expected to be related to the extent to which his or her disease process is dominated by the autoimmune attack at the site represented by this synthetic peptide. Results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment showed that intravenous MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive MS (Warren et al. 2006). A pivotal phase II/III clinical trial is in progress. MBP8298 can be considered as a personalized treatment of MS.

Pharmacogenomics of IFN-β Therapy in MS

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 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 MS 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 interferon beta, the work has the potential to change the approach to MS treatment from a hit-and-miss one to a more systematic personalized management.

The BENEFIT (BEtaseron/Betaferon in Newly Emerging MS 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 MS (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 of response to IFN-β

therapy in MS 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 (Martinez-Forero et al. 2008).

Understanding of the factors that underlie the therapeutic response is key to the 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. Ultimately, this information will lead to personalized therapy for MS (Vosslamber et al. 2009).

Future Prospects of Personalized Therapy of MS

In the near future, studies on susceptibility genes and pharmacogenetics will provide invaluable information concerning new drugs for the treatment of MS and better therapeutic regimens for these patients. Future approaches to MS 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.

Personalized Management of Psychiatric Disorders

Psychopharmacogenetics

Variability of the drug response is a major problem in psychiatry. Between 30–50% of the patients do not respond adequately to initial therapy and it can take several months to find this out. A study of the pharmacogenomic and pharmacogenetic basis of these disorders is important.

Most psychiatric disorders, including schizophrenia, major depression, and bipolar disorder, are considered polygenic. 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) 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), an 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.

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, IL), 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.

COMT Genotype and Response to Amphetamine

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. A functional polymorphism (val158-met) in the catechol *O*-methyltransferase (COMT) gene has been shown to modulate prefrontal dopamine in animals and prefrontal cortical function in humans. Amphetamine enhanced the efficiency of prefrontal cortex function assayed with functional MRI during a working memory task in subjects with the high enzyme activity valve genotype, who presumably have relatively less prefrontal synaptic dopamine, at all levels of task difficulty (Mattay et al. 2003). In contrast, in subjects with low activity met/met genotype who tend to have superior baseline prefrontal function, the drug had no effect on cortical efficiency at low-to-moderate working memory load and caused deterioration at high working memory load. These data 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.

Genotype and Response to Methylphenidate in Children with 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 is often based on trial and error. Stimulant medications, such as methylphenidate are the most commonly used, effective treatment for 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 DAT 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 in progress to evaluate children with ADHD on two other medications to see if their genes predict who will respond to either or both drugs.

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 approximately 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.

A 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 test 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 a significant decrease in clozapine clearance compared with wild-type mice and the prolonged half-life of plasma clozapine suggests 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, several dopamine, serotonin, histamine, and adrenergic receptor polymorphisms have been studied. A combination of receptor polymorphisms can predict antipsychotic medication response. 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 will also be applied to other antipsychotic medications. In future, simple psychopharmacogenetic tests will improve antipsychotic medication treatment and its application among individuals.

The ability of dopamine receptor polymorphism to predict clinical response to clozapine has been studied using PET. Studies with PET using FDG and dopamine D3 receptor polymorphism in the promoter region for genetic association 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 the clinical response to treatment and may predict side effects.

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. 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 11.1. 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.

ACADIA Pharmaceuticals is collaborating with the Karolinska Institute (Stockholm, Sweden) to examine possible genetic variations in schizophrenic patient populations that may contribute to differential responses to atypical and typical (i.e., clozapine and haloperidol, respectively) antipsychotic drugs. ACADIA's proprietary technology, a massively parallel, drug discovery engine, is called

Table 11.1 Enzymes that metabolize antipsychotics

Drug	CYP2D6	CYP2C19	CYP3A4	CYP1A2
Chlorpromazine	+			
Clozapine	+		+	+
Fluphenazine				+
Haloperidol	+		+	+
Olanzapine		+	+	
Perphenazine	+			
Risperidone	+			
Sertindol	+			+
Thiorodazine	+	+		
Zuclopentixol	+			

Receptor Selection and Amplification Technology (R-SAT). Once the contributing factors to genetic variation in drug response are determined from these and other studies, a pre-emptive strike can be initiated. 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.

Nanogen acquired rights to genetic biomarkers related to schizophrenia and responses to antipsychotic therapies from the Co-operative Research Centre for Diagnostics and Queensland University of Technology in Australia. Nanogen plans to utilize the biomarkers to create diagnostic tests for schizophrenia and related conditions. Some of these biomarkers may also help predict adverse drug reactions (ADR) and therefore guide therapeutic decision-making.

ADRs to antipsychotic therapy constitute another area of concern. The CYP2D6 poor metabolizer phenotype appears to be associated with risperidone ADRs and discontinuation due to ADRs. 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.

Resperidol'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 ADRs 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 ADRs. 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

After multiple trials, approximately 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. Pharmacogenomic approaches could help in predicting some of these outcomes. Enzymes that metabolize antidepressants are shown in Table 11.2.

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 for the therapeutic effects of antidepressants, a new antidepressant pharmacotherapy could be developed to activate that system more acutely. A 5-HT_6 receptor polymorphism (C267 T) is associated with treatment response to antidepressant treatment in major depressive disorder (Lee et al. 2005). A pharmacogenomic approach to individualize antidepressant drug treatment should be 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

Table 11.2 Enzymes that metabolize antidepressants

Drug	CYP2D6	CYP2C19	CYP3A4	CYP1A2
Amitripyline	+	+	+	+
Nortriptyline	+			
Imipramine	+	+	+	+
Desipramine	+			
Clomipramine	+	+	+	+
Citalopram		+	+	
Fluoxetine	+			
Fluvoxamine	+			+
Moclobemid		+		
Paroxetine	+			
Sertraline			+	
Venlafaxine	+		+	

Although personalized medication that is based on pharmacogenomic/pharmacogenetic data is expected to improve the efficacy of treatments for depression, the complexity of the regulation of gene transcription and its interactions with environmental factors implies that straightforward translation of individual genetic information into tailored treatment is unlikely. However, integration of data from genomics, proteomics, metabolomics, neuroimaging, and neuroendocrinology could lead to the development of effective personalized antidepressant treatment that is based on both genotypes and biomarkers (Holsboer 2008).

Pretreatment EEG to Predict Adverse Effects to Antidepressants

Changes in brain activity prior to treatment with antidepressants can flag patient vulnerability. Quantitative electroencephalography cordance 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 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.

Individualization of SSRI Treatment

The introduction of the selective serotonin reuptake inhibitors (SSRIs) 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; as 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 the 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 the 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. Because 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-methylenedioxymethamphetamine (MDMA, or Ecstasy), a serotonin transporter, is associated with higher depression scores owing 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, which identifies people who respond differently to antidepressants, including SSRI. SSRIs act specifically by binding to the serotonin transporter, and increase the concentration of the neurotransmitter serotonin in the synapse. These medications include fluoxetine, sertraline, paroxetine, citalopram, and escitalopram.

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, namely, 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.

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 and Eliasson. 2007). TDM combined with genotyping of CYP2D6 is particularly useful in verifying concentration-dependent 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 because of UM may be mistaken for poor compliance with the prescription.

Vilazodone with a Test for Personalized Treatment of Depression

Vilazodone (Clinical Data Inc.), 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. In 2007, the primary and supportive secondary efficacy endpoints were met in the randomized, double-blind, placebo-controlled trial. In addition, the study separately identified candidate biomarkers for a potential companion pharmacogenetic test for response to vilazodone.

Summary

Personalized neurology requires the integration of several neuroscientific and clinical aspects of neuropharmacology. Molecular imaging is important for CNS drug discovery and development. The pharmacogenomics of neurodegenerative disorders 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.

Despite numerous AEDs in the market, treatment of epilepsy is unsatisfactory. Gene mutations are being identified in epilepsy, e.g., those in ion channel genes. Future drugs may be designed specifically according to the electrophysiological dysfunction as personalized medicines for epilepsy. The wide heterogeneity of MS and the different biological responses to immunomodulatory drugs contribute to different treatment results. Considerable efforts are under way to personalize treatment of this disease. In the near future, studies on susceptibility genes and pharmacogenetics will provide invaluable information concerning new drugs for the treatment of MS and better therapeutic regimens for these patients. This chapter also considers the personlization of psychiatric treatment particularly that involving antipsychotics and antidepressants.

Chapter 12
Personalized Therapy of Cardiovascular Diseases

Introduction

The constantly growing volume of available data on cardiovascular disorders will require an organized interpretation of variations in DNA and mRNA as well as proteins, both on the individual and the population levels. 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

Role of Cardiovascular Diagnostics in Personalized Management

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

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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 on 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 (CRP), a marker 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 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 compared to 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.

SNP Genotyping in Cardiovascular Disorders

Scientists at the Joslin Diabetes Center (Boston, MA) have invented diagnostic methods to detect an individual's susceptibility to developing cardiovascular disease by analysis of specific SNPs within the receptor gene that correlate to the disease risk. Two specific SNPs were analyzed and found to correlate to the risk of coronary artery disease (CAD) in two specific populations. Minor allele homozygotes for one of the SNPs had more than a twofold increase in CAD risk across both populations. Homozygotes for a particular haplotype of the other SNP were 1.7-fold more likely to have had a myocardial infarction (MI). In addition, homozygotes for the first SNP showed 30% lower levels of mRNA for the receptor. The invention therefore features methods of diagnosing or detecting susceptibility to cardiovascular disease by typing specific SNPs in the genome of an individual.

Common SNPs at 18 loci are reproducibly associated with concentrations of low-density lipoprotein (LDL) cholesterol, high density lipoprotein (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 CAD. 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.

Cardiovascular Disorders with a Genetic Component

Genetic testing can be effectively used to distinguish between heart failure patients who suffer from ischemic or nonischemic forms of the disease. Johns Hopkins scientists have used groupings or clusters of a patient's gene expression to compare to a diseased "test" set that identifies the cause of heart failure. Using a biostatistical technique of prediction analysis, the investigators have identified the 90 genes that best distinguished the two kinds of heart failure. The large number of genes used also improved accuracy of the test. Results showed the test profile to be highly accurate, with 90% specificity. The findings could, if confirmed and adapted to a standardized and affordable test format, someday aid physicians in the diagnosis of heart failure and help determine which kind of therapy is best to use for the condition.

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 12.1.

Long QT 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™ (Clinical Data Inc.) is the first commercially available, comprehensive

Table 12.1 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
Hypertension	Essential hypertension	AGT	Contraction of arterial smooth muscle
Blood lipid disorders	Familial hypercholesterolemia	LDL	Regulation of LDL
	Familial dyslipoproteinemias	ApoE	Regulation of plasma lipid concentrations
Atherosclerosis	CAD	E-S128R	Monitors white blood cell adhesion to the arterial wall
Atherosclerosis	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 APC
	Stroke		

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 have 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 they carry the familial mutation.

Gene Variant as a Risk Factor for Sudden Cardiac Death

The extremes of the electrocardiographic QT interval, a measure of cardiac repolarization, are associated with increased cardiovascular mortality. A gene called NOS1AP, 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 QT interval length significantly, as a 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 with a long or short QT interval. Only one particular SNP correlated with the 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.

SNP Chip for Study of Cardiovascular Diseases

Illumina is developing 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, will be used to analyze more than 55,000 SNPs in genes that have been selected for cardiovascular-related phenotypes. The collaborators will use the Illumina iSelect Custom Genotyping BeadChip to study the genetic diversity of pathways for around 2,100 genes that are linked to vascular conditions including hypertension, MI, 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 study plans to analyze more than 120,000 samples from population studies and clinical trials for possible links to vascular 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.

Pharmacogenomics of Cardiovascular Disorders

Application of pharmacogenomics for development of personalized treatment of cardiovascular disorders is illustrated by a few examples, such as MI, 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 MI

Variants in the 5-lipoxygenase-activating protein (FLAP) gene are associated with risk of 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.

Management of Heart Failure

A large number of drugs are used in the management of heart failure. Examples relevant to personalized medicine will be considered here: β-blockers, Bucindolol, and BiDil.

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 because of hypotension, bradycardia, and worsening of heart failure. This has led to the study of genetic variants that determine the 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 the drug target, the β_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 β_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 is used in advanced heart failure patients 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 β1-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure. A study concluded that β1AR-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 US heart failure population. Bucindolol's unique pharmacology gives it other advantages as well, such as superior MI 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 is 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.

Management of Hypertension

Hypertension is a common disorder affecting approximately 20% of the US population. Care of hypertensive patients vary a lot. Ideally, individual risks must be assessed 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. Once the decision to treat has been made, an appropriate therapy should be selected.

Approximately 100 medications are available for treatment in several categories: diuretics, α-blockers, β-blockers, aldosterone antagonists, 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 hypertension. However, as many as 60% of hypertensive patients do not achieve adequate blood pressure lowering from monotherapy with β-blockers. It is plausible that genetic variation in the β-adrenergic-receptor genes account for some of the observed variability in blood pressure response.

Pharmacogenomics of Diuretic Drugs

Diuretics are considered to be first-line drugs for hypertension 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), cytochrome P450 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 such as 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 the 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, whereas 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 hypertension, 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. Hypertension 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 of 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 efficacious drug as first line of treatment leading to reduction of the number of drugs required for adequate treatment and 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.

Management of Hypertension by Personalized Approach

Despite the many therapeutic options for hypertension, only 27% of the patients achieve adequate control of blood pressure. Therefore, there is an opportunity to improve the management of hypertension through a personalized approach as shown in Fig. 12.1.

Being a polygenic disorder, hypertension still remains a challenge for designing better future treatments. The largest and most recent searches of the genome have found limited evidence of genes that determine hypertension. Linkage analysis identified a principle locus on chromosome 6q, with a lod score of 3.21 that attained

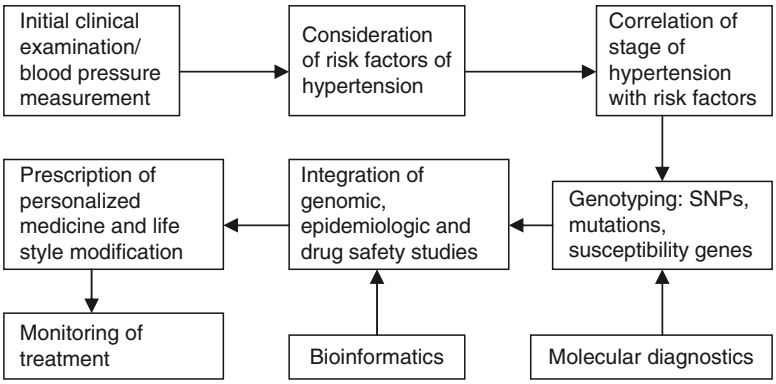

Fig. 12.1 A scheme of personalized approach to management of hypertension. ©Jain Pharma-Biotech

genome-wide significance (Caulfield et al. 2003). The discovery of a single allele proven to be associated with control of blood pressure could lead to the discovery of relevant and novel targets for prevention and treatment of hypertension.

Pharmacogenetic-guided therapy has clinical potential for management of hypertension, but there are few controlled studies on this topic. A clinical trial on individuals with uncomplicated hypertension aims to identify the genetic determinants of the antihypertensive and adverse metabolic responses to a thiazide diuretic (hydrochlorothiazide), a beta-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 hypertension 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.

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 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 hypolipemic 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 and Muallem 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 LDL cholesterol (LDLc) lowering by statins. An intronic SNP in ABCA1 and the apolipoprotein E (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 endothelial nitric oxide synthase (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 CAD and MI. However, contradictory results have also been reported. One study has investigated the associations of eNOS polymorphism with these diseases in two prospective autopsy series comprising altogether 700 Caucasian Finnish men who died suddenly (Kunnas et al. 2002a). In ANCOVA, 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 MI 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 MI. In a placebo-controlled study, adenosine-stimulated myocardial perfusion, as determined by PET, improves after treatment with pravastatin in subjects with the eNOS ba-genotype but not in those with the bb-genotype (Kunnas et al. 2002b). 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.

The STRENGTH Study

The STRENGTH study (Statin Response Examined by Genetic HAP Markers) of 2001 is the largest prospective clinical trial ever conducted to discover how physicians can personalize prescriptions using information about human genomic variation. As the earliest application of pharmacogenomics to one of the most prevalent public health problems – hypercholesterolemia – the study was designed to provide the information necessary for physicians to decide which cholesterol lowering drug is best for each patient based on their own genetic make up. The four drugs under study were atorvastatin, simvastatin, pravastatin, and cerivastatin.

In 2002, STRENGTH I clinical study that further demonstrated the ability of its HAP Technology to identify specific genetic markers (gene haplotypes or HAP Markers) that are associated with the effects of statin therapy, including LDL (bad) cholesterol, HDL (good) cholesterol and triglycerides. Twenty-five of the markers were linked to outcomes for specific drugs and four were associated with the effects of statins as a drug class. These important findings highlight the differences between drugs in the statin class and clearly indicate the need and the potential to optimize therapy based on the genetics of different patient populations. The medical community has been aware of clinical and metabolic differences among the statins but this study provided some genetic evidence that begins to explain these differential effects. Further clinical studies have continued.

Marked lowering of LDLc levels (< or = 50%) with intensive statin therapy is associated with major reduction in cardiovascular risk, but is limited by a potential increase in adverse effects, thereby justifying optimization of LDLc 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 hypercholesterolemai reveal that the 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.

Personalized Management of Women with Hyperlipidemia

A study conducted by Genaissance Pharmaceuticals (now taken over by Clinical Data Inc.) on individuals, who were candidates for statin therapy, suggests that women with a genetic predisposition to protective levels of CRP (C-reactive protein, an established marker for fatal coronary disease) lose that benefit when taking hormone replacement therapy (Judson et al. 2004). The results of this study are the first published results from Genaissance's STRENGTH study described in the preceding section.

Several studies by leading academic/medical centers have shown that 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 hormone replacement therapy (HRT) caused elevated levels of CRP and of heart attacks and strokes (Women's Health Initiative). The current study shows that the protective effect of a key genetic variant may be overwhelmed by the use of these drugs.

The results give lifestyle guidance to women who would like to preserve the protective benefits conferred by favorable genetic variations, and may ultimately lead to new or modified drugs. The study showed that men and women with common variants in the ApoE gene on average have naturally lower levels of CRP. In the case of women, however, the study indicates that this beneficial effect may be largely neutralized by HRT, allowing CRP levels to potentially increase to dangerous levels.

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 MI 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 therapy 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 (Gage et al. 2004). In a study of orthopedic patients, Gage showed that testing for CYP2C9 polymorphisms does provide 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. Mutations in one of the complex's subunits, VKORC1, confer warfarin resistance in some human disorders. Overexpression of the wild-type protein made rats

sensitive to the treatment. Future studies will genotype for both CYP2C9 and VKORC1 when prescribing warfarin before surgery.

Heparin is used to prevent and treat thromboembolic diseases. One of the most serious adverse reactions to heparin is an immune-related thrombocytopenia. Heparin-induced thrombocytopenia (HIT) 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 single-nucleotide polymorphism 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.

Nanotechnology-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 (Wickline et al. 2006). 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 in this situation. Similar behavior has been demonstrated for doxorubicin containing particles. Intravenous delivery of fumagillin (an antiangiogenic agent)-loaded nanoparticles targeted to $\alpha v\beta 3$-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 Cardiovascular Diseases

In August 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. euHeart project complements the earlier HeartCycle project, which focuses on the long term management of chronic heart disease patients. The euHeart consortium, led by Philips Healthcare, aims 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 computed tomography and MRI scans, measurements of blood flow, and blood pressure in the coronary arteries and ECGs. These computer models will integrate the behavior of the heart and the aorta at molecular, cellular, tissue, and organ-level. They will also incorporate clinical knowledge about how cardiovascular disease disturbs the correct functioning of the heart at these levels. 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.

As an example, one way of treating heart rhythm disorders is 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 able to test the results of destroying different areas of tissue before operating on the patient.

Concluding Remarks

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 MI and stroke when treated with diuretics compared with other antihypertensive therapies. With regard to blood pressure 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 the response to antihypertensive drugs. Findings from previous studies require confirmation from other studies to be able to come to 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.

Summary

There are already applications of pharmacogenomics for development of personalized treatment of cardiovascular disorders as illustrated by a few examples, such as MI, heart failure, and hypertension, which are common conditions. Hypertension a polygenic disorder with over 100 drugs from several categories that are available for treatment provides challenges in management. Pharmacogenomics of antihypertensive drugs is discussed and a scheme for personalized treatment is presented. 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 the response to antihypertensive drugs.

Chapter 13
Personalized Management of Miscellaneous Disorders

Management of Viral Infections

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. The main example is that of HIV infection.

Management of HIV

There are two variable factors in HIV/AIDS – how people respond to the HIV and how HIV responds to drugs. Research in vaccinology is playing an important role in relation to immunology of HIV/AIDS.

Genetics of Human Susceptibility to HIV Infection

Humans are not equal in terms of susceptibility to HIV infection, or in the rate of disease progression. This is evidenced by the identification of individuals who remain seronegative despite multiple exposures to HIV-infected partners, and by the existence of the so called "long-term progressors". Currently used research approaches include:

- Analysis of the differences in 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 setpoint after HIV seroconversion. This is a collaborative European project supported by the Center of HIV/AIDS vaccine immunology/NIH (CHAVI).

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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 was 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. The work will provide a unique description of how the host's 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 the breadth 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 the body's response 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.

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 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 investigate the basis of metabolic and lipid disorders associated with the 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.

Role of Diagnostic Testing in HIV

The 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

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.

Drug-Resistance in HIV

Although antiretroviral drugs are highly effective in reducing viral replication and have significantly reduced death rates from the AIDS in the USA, drug resistance threatens their utility. Despite the availability of over numerous anti-HIV drugs, up to 50% of the patients on combination therapy experience treatment failure mainly due to development of resistance to the drugs. The rational selection of combinations of drugs to avoid or overcome resistance is one of the critical challenges in achieving long-term viral suppression and optimal clinical outcome in HIV/AIDS. The cause of resistance is extremely complex, because over 100 individual mutations in the HIV genetic code are known to be involved. The following indicates clinical utility of genotyping:

- Drug resistance mutations are independent markers of virologic failure.
- Treatment failure does not indicate failure of all drugs in a combination.
- Provides information about cross-resistance.

Assays for drug resistance testing in HIV-1 infection are now available and clinical studies suggest that viral drug resistance is correlated with poor virologic response to new therapy. The clinical utility of genotyping has been established. Emerging data indicate that despite limitations, resistance testing should be incorporated into patient management in some settings. Resistance testing is recommended to help guide the choice of new regimens after treatment failure and for guiding therapy for pregnant women. It should be considered in treatment-naive patients with established infection, but cannot be firmly recommended in this setting. Testing also should be considered prior to initiating therapy in patients with acute HIV infection, although therapy should not be delayed pending the results. Expert interpretation is recommended given the complexity of results and assay limitations.

PhenoSense HIV (Monogram Biosciences) is a rapid, sensitive, and comprehensive phenotypic drug susceptibility assay for HIV-1 that directly measures the susceptibility, or resistance, of a patient's virus to all currently available antiretroviral drugs (reverse transcriptase and protease inhibitors). HIV replication capacity, as measured by the PhenoSense HIV assay, may be an additional predictor of clinical outcome and may complement other laboratory parameters, such as viral load and CD4 cell counts, in making individualized antiretroviral treatment decisions, especially for patients experiencing failure of their treatment regimen.

Measurement of Replication Capacity

HIV-1 uses the CD4 cell surface receptor and one of two co-receptors (CCR5, CXCR4) to infect cells. A switch from the CCR5 to the CXCR4 co-receptor is associated with more rapid disease progression and death from AIDS related illness. Replication Capacity (Monogram Biosciences) provides an important measure of the ability of HIV to proliferate and is currently offered with Monogram Biosciences' PhenoSense and PhenoSense GT. Genetic changes (mutations) in HIV that confer drug resistance often impair the virus' ability to replicate efficiently and lead to reductions in replication capacity (RC). Several clinical studies have found that patients experiencing treatment failure do not progress to AIDS if the drug resistant virus has impaired replication capacity. These findings support the use of RC measurement as a tool for the management of HIV infection and to help individualize treatment regimens. A follow up study has demonstrated that the emergence of CXCR4 virus variants independently predicts immune system deterioration and HIV disease progression.

Prevention of Adverse Reactions to Antiviral Drugs

Efavirenz is commonly a component of drug cocktails used to fight HIV, but it can cause neurological adverse reactions such as disturbing dreams and dizziness. A genetic mutation in the gene for CYP2B6, which occurs in 20% of blacks but

only 3% of whites slows the drug metabolism and nearly triples the average blood concentration of the drug (Andrade and Flexner 2004). -This increases the odds that people taking efavirenz will suffer side effects that lead them to discontinue the treatment. Patients with this mutation should start therapy with a low dose of efavirenz. Another factor that affects the drug metabolism is body weight. Heavier persons tend to metabolize efavirenz relatively quicker than those with lower weights. Patients who clear the drug very rapidly may show lack of efficacy of the drug. Personalized approach to therapy would take these variations into consideration. Genotyping could predict the response to therapy regardless of the racial difference.

QIAGEN has introduced a SSP© PCR assay to type the HLA-B*5701 allele, a genetic variation in the HLA system. HIV patients carrying the HLA-B*5701 marker have a 60% higher risk to develop hypersensitivity reaction (HSR) to Abacavir, which is a component of several marketed drugs inhibiting the reverse transcriptase of HIV. HSR is a serious and sometimes even fatal multi-organ syndrome that manifests by fever, respiratory or constitutional symptoms. The FDA has already advised healthcare professionals that all HIV patients should be screened for HLA-B*5701 before initiating treatment with drugs containing Abacavir. Health authorities in other countries have issued similar warnings in response to the PREDICT1–1-Study, which found that HLA-B*5701 is a major biomarker for the HSR (Ingelman-Sundberg 2008). The screening for HLA-B*5701 prior to Abacavir treatment allows the identification of patients likely to develop HSR. Using HLA-B*5701 tests as a companion diagnostic with the drug Abacavir therefore helps to better protect HIV-infected patients in treatment from severe additional suffering. The combination of diagnostics and therapeutics is a key approach to eliminating risks of side effects and therefore increasing the efficacy of drugs.

Role of Genetic Variations in Susceptibility to HIV-1

In 2008, the NIH was supporting research projects to study genetic variations linked to susceptibility for HIV-1 infection and AIDS progression among drug-abusing populations. It will also support research into the effects of viral mutations and recombination associated with drug abuse on host responses to infection, as well as the pharmacogenetics of interactions among HIV-1 treatment medications and either drugs of abuse or therapies used in the treatment of drug addiction. The research would involve individuals chronically using addictive substances, or use of appropriate in vitro or in vivo models, in order to improve our understanding of the role of genetic variation within genes involved in modulating immune function, or genes that are highly expressed in monocyte derived dendritic cells, mucosal cells, or other cells/tissues that may alter an individual's susceptibility to HIV-1 infection. NIH also plans to study whether drugs of abuse such as methamphetamine interact with host or viral genetic factors to either increase HIV-1 susceptibility or diminish the host's ability to internalize pathogens and subsequently activate T cells.

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 (Tozzi et al. 2008). Studies across the world have consistently demonstrated that HLA-B*5701 predicts the likelihood of HSRs 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. Moreover, 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 hyperlipidaemia, 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.

Treatment of Hepatitis B

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 the response to IFN. With the increasing progress in nucleic acid technologies, investigation of viral genetic markers may soon be integrated in clinical diagnostic routine.

Treatment of Hepatitis C

Hepatitis C is the most common blood-borne viral infection in the USA and it is one of the main causes of chronic liver disease. It is estimated that at least 4 million persons in the USA and 170 million persons world-wide are infected with hapatitis C virus (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.

Standard treatment for hepatitis C, weekly injections of IFN and the oral antiviral agent ribavirin can be curative, but only ~40% of patients with the most common subtype of HCV in the USA, genotype 1, will respond to it, and it is not clear who is likely to respond and who is not. The result is that thousands of people spend long months on treatment without any significant long-term benefit. 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 pretreatment indicators of sustained virological response. The most reliable predictor of a poor virological response is continued seropositivity for viral RNA during therapy. Genelyzer (Toshiba Corporation), an electrochemical DNA chip, has been used to detect resistance to treatment in patients with hepatitis C. Celera Genomics has developed a genetic test that can help predict which patients with hepatitis C will eventually develop cirrhosis and so are in most need of treatment. The test, which looks at variations in seven genes, will help to personalize treatment.

The genomic sequences of independent HCV isolates differ by approximately 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 greater than 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.

To predict the response of HCV to IFN/ribavirin treatment, researchers at the Duke Clinical Research Institute (Kannapolis, NC) selected serum samples from patients with genotype I who responded to therapy and were cured; from patients with genotype I who did not respond to therapy and also from patients with genotypes 2 or 3 who had also responded to therapy and were cured. They broke down the proteins in the serum into peptides and then used LC/MS to sort the peptides according to molecular weight and charge. Using factor modeling in conjunction with software designed to analyze proteomic data (Rosetta Elucidator), they discovered three factors representing clusters of proteins or peptides that can predict in

9 cases of out 10 who will respond to therapy and who will not. Further investigation will be done to determine the protein pathways these clusters are associated with, which may yield information that could lead to new treatment options or more informed treatment decisions using current therapies. These protein signatures will be investigated in a planned clinical trial.

A 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 peg IFN 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.

Personalized Management of Tuberculosis (TB)

TB is a global pandemic that threatens to overwhelm healthcare budgets in many developing countries. It is estimated that at least 8 million people develop active TB annually, of whom 2 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 TB 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 TB 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. 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 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 creams. The latest approach developed by Lab 21 (New York) claims that by taking DNA samples from customers it can provide a personalized skin cream based on specific variations of the 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 when we do not know which genes are regulating skin care is unscientific. Another issue is privacy. On the swabs the consultants take at the shops is a complete set of an individual's genetic information. A lab could tell whether a person had genes for all sorts of 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 has invented the first genetically designed patentable DNA test for customized skin-care products, and in partnership with DNAPrint, the companies anticipate screening millions of candidate markers. Tests are designed to assess genetic risks for certain skin and nutritional deficiencies and provide a basis for recommending formulations that have been specifically designed to compensate for these deficiencies.

Personalized Therapy of Rheumatoid Arthritis (RA)

RA is a multicomplex system inflammatory disorder, which affects the synovial lining of the joints and tendons. The cause of RA is not known but both inherited and environmental factors are generally considered to play a role with systemic immune reactions precipitating a cascade of inflammatory reactions.

Hyperproduction of interleukin-6 (IL-6) is observed in RA patients and the 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).

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 RA. 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 for patients with RA. Some published data suggest that a patient's IL-1 genotype may predict his or her response to drug therapy.

Pharmacogenomic studies on MTX, sulfasalazine, and TNF-α inhibitors have been reported, suggesting that the pharmacogenomic approach may be useful for the treatment of RA. Although there are 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).

Cypress Bioscience Inc, by acquisition of Proprius Pharmaceuticals Inc in March 2008, is developing personalized therapy for RA. An early RA prediction technology will be used to determine the likelihood of developing RA in patients

with undifferentiated arthritis. A MTX polyglutamates monitoring assay will help physicians to optimize MTX therapy by providing insights on an individual's metabolism of MTX.

DIATSTAT™ Anti-Cyclic Citrullinated Peptides in RA

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' RA 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 versus RF. 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 RA. 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 neurogenerative 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 RA. 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 RA 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 (Brendtzen 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 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 which 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). Only a few therapeutic agents based on novel mechanisms of action have been developed over the past two decades. 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.

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).

Genetic Polymorphism and Response to β_2-Adrenergic Agonists

Inhalation of salbutamol, a β_2-adrenergic agonist that has a bronchodilator effect in asthma, aids the flow of air to the lungs. β_2-adrenergic receptor gene contains 13 SNP and an analysis of all the possible interindividual variations shows that four

common differences predict how people would respond to salbutamol. This drug worked 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 β_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 β_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 β_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.

Genotyping in Asthma

Recent studies show that 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 individuals 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 to suit the genetic make-up of an individual.

Orchid, GeneShield, and Merck-Medco are collaborating to conduct a retrospective, observational health outcomes study combining pharmacy, medical claims, and genotyping data for 2,000 participating managed care patients with asthma. The study will focus on assessing the impact of a relatively common genetic variation on clinical outcomes and health care resource utilization for patients using drugs commonly employed for the management of asthma. The results of the study

are expected to provide preliminary data indicating whether physicians should consider alternative regimens to better manage those asthma patients having the genetic variation.

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.

Personalized Approaches in Immunology

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.

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.

Pharmacogenetics and Pharmacogenomics of Immunosuppressive Agents

Immunosuppressive therapy has markedly improved over the past years with the advent of highly potent and rationally targeted immunosuppressive agents. Because 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 present several limitations. Pharmacogenomics might represent a complementary support. Preliminary studies that have focused on polymorphisms of genes encoding enzymes involved in drug metabolism, drug distribution, and pharmacological target, have shown promising results. Pharmacogenomics holds promise for improvement in the ability to individualize pharmacological therapy based on the patient's genetic profile.

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 immunosuppressant 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, RA 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.

DNAPrint genomics Inc entered into a collaboration with the New York University School of Medicine (New York) to develop pharmacogenomic classifiers for organ transplant patients. Using qualified patient specimens and matching clinical data, DNAPrint genetically screens the specimens for markers and/or marker sets that can be used to distinguish between drug responders and non-responders. The goal is to identify pharmacogenomic classifiers that could be used to match renal transplantation patients with the optimal immunosuppressant for their genetic make-up.

Personalized Management of Pain

Interindividual differences in the experience of pain have been appreciated clinically for over a century. A scheme of personalized management of pain is shown in Fig. 13.1.

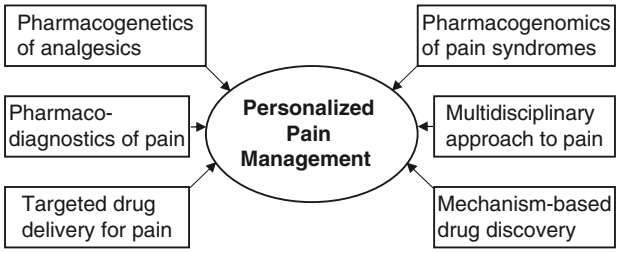

Fig. 13.1 A scheme of personalized management of pain. © Jain PharmaBiotech

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. 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 oftentimes 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 (Dionne et al. 2005).

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. One study has investigated genotype, phenotype, and morphine production from codeine in children undergoing adenotonsillectomy and compared analgesia from codeine or morphine combined with diclofenac (Williams et al. 2002). The conclusion was that reduced ability for codeine metabolism may be more common than previously reported. Plasma morphine concentration 1 h after codeine was related to phenotype and very low.

Codeine analgesia was less reliable than morphine but was not well correlated with either phenotype or plasma morphine in this study.

Although morphine is the analgesic of choice for moderate to severe cancer pain, 10–30% of patients do not tolerate morphine. A study evaluated genetic variation in the mu-opioid receptor in patients who responded to morphine versus those who were switched to alternative opioids. The data suggest that variation in genes involved in mu-opioid receptor signaling influences clinical response to morphine (Ross et al. 2005).

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 in 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 (Woolf 2004). 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 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 their 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 anytime. 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 more 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 RA may receive different medications.

Management of Genetic Disorders

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 Chapter 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. Cystic fibrosis is used as an example.

Personalized Treatment of Cystic Fibrosis

Cystic fibrosis is the most common serious genetic disease among Caucasians in the United States. The disease results from a defective gene that affects multiple aspects of cellular function. Its most serious symptom is a build-up of thick, sticky mucus in the airways, which can lead to fatal lung infections. 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 cystic fibrosis 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 medical treatment. Methods used currently for the treatment of pulmonary complications of CF include physiotherapy, bronchodilator therapy, mucolytic agents and corticosteroids. Many of these therapies are individualized according to the needs of the patients, which vary considerably. Lung transplant is the last resort for advance pathology. These methods are directed at the management of manifestations and none of these addresses the cause of the disease. Because of the devastating clinical sequelae and the lack of definitive therapy, 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 drug 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.

Personalized Management of Gastrointestinal Disorders

Personalized Therapy of 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 USA 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 USA and Europe. IBD patients are treated by sulfanomides, 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).

Advancement of genome analysis might have an impact on the treatment of IBDs. Genomic studies have revealed some genetic factors contribute to pathogenesis of IBD such as HLA, IL4, MUC3, IBD1 locus, IBD2 locus (Takei et al. 2001). 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.

Crohn's disease is characterized by variation in both location and behavior. Chromosome 16 and the HLA region on chromosome 6 have been implicated in susceptibility to disease. Mutations in the NOD2/CARD15 gene, recently identified on chromosome 16, have been associated with disease overall but are found in only 25% of patients. The clinical pattern of Crohn's disease may be defined by specific genotypes. This study may provide the basis for a future molecular classification of disease.

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 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 marker 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 Approach to Addiction

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. However, alcoholism represents a complex psychiatric disorder, which is 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. New treatment strategies focusing on genes contributing to drug and alcohol dependence (such as gene therapy) are already under examination in animal models. However, further research is required before these developments will considerably change today's clinical handling of alcoholism on an individual basis.

Personalized Therapy for Smoking Cessation

The evidence to date is very consistent with respect to the significance of genetic contributions to smoking behavior. However, attempts to elucidate the role of specific genetic variants have met with mixed success. Explanations for the lack of consistency in the results of genetic association studies include biases in ascertainment, ethnic admixture, lack of attention to co-variates or modifiers of genetic risk, and the need for more refined phenotypes. 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. Now researchers have demonstrated that a dopamine receptor gene polymorphism appears to influence the response of cigarette smokers to smoking cessation therapy that includes an antidepressant medicine – venlafaxine. This study is being conducted at the University of Texas MD Anderson Cancer Center. Researchers genotyped 134 smokers to determine whether they carried the A1 or A2 allele of the DRD2 gene. Individuals with at least one copy of the A1 allele of the gene have fewer and less-sensitive D2 dopamine receptors than do individuals with two copies of the A2 allele. As part of a smoking-cessation protocol, half the smokers were given venlafaxine whereas the other half received a placebo. All the smokers were offered standard smoking-cessation counseling and transdermal nicotine. The researchers found 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 reported 25% lower score on testing for negative affect. This study 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.

Personalized Approach to 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 (Rutter 2006).

The National Institute of Drug Abuse (NIDA) was seeking information about SNPs to include in a custom microarray platform it is designing 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.

Personalized Approaches to Miscellaneous Problems

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-alpha) 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. Several clinical trials have been conducted to study this in recent years. 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 findings point to the possibility of using genetic screening to tailor decisions about hormone-replacement therapy to maximize the health and well being 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 Treatment of Malaria

World wide there are an estimated 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 its efficacy in most parts of the world. There are few effective treatments available. Verpamil, when given in combination with chloroquine, 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*.

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 the 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 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 have an effect. 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 drug therapies.

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 (Rudnicki and Mayer 2003). 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 hyperoxaluriã, 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 (Monico et al. 2005). 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.

Personalization of Organ Transplantation

Two examples of typical organ transplants, kidneys and heart, will be used to illustrate the personalized approach to 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 more than half of the biopsies taken from kidney transplant patients who appeared to be doing well only 2 years after transplantation show signs of chronic allograft nephropathy. 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.

A research project titled "Genomics for Kidney Transplantation," and funded by the National Institute of Allergy and Infectious Diseases (part of the NIH) will study some 2,400 patients with kidney transplants to find out the genetic basis and control of why some patients do well and others have problems. It started in 2004 and more than $12 million have been spent over the following 5 years to apply cutting-edge genomic technologies to advance the understanding of kidney transplantation. Investigators are monitoring several hundred patients who have had kidney transplant surgeries with technologies for gene expression profiling and proteomics, and several thousand transplant patients by complex trait genetics.

Personalization of Cardiac Transplantation

The Cardiac Allograft Rejection Gene Expression Observational study (CARGO) was initiated by XDx Inc in 2001 to study the utility of peripheral blood gene expression for cardiac transplantation acute rejection management. The study also evaluated gene expression testing in relation to clinical endpoints such as the development of graft dysfunction and the need for anti-rejection therapy. Eight leading US transplant centers, which represent more than 20% of the yearly cardiac transplant volume in the USA, participated in the study. Over 600 cardiac transplant recipients are enrolled in the study and have been followed during their post-transplant course consisting of over 5,000 clinical encounters.

Using a 'genome-wide' approach, XDx developed a leukocyte gene library consisting of over 8,000 genes known to be involved in immune responses. Select sequences representing these genes were incorporated into custom microarrays and

used to examine gene expression in the CARGO study. Patient blood samples were obtained at the time of biopsy and the expression levels of these 8,000 genes were ascertained and compared to the biopsy result. A subset of over 200 candidate genes showed promise as markers which discriminate rejection from quiescence. The next phase of the CARGO study used the more specific and sensitive real-time PCR technology to measure gene expression levels of more than 200 genes. These studies provided highly quantitative and reproducible measures of expression levels for each gene.

The CARGO study resulted in the identification and validation of gene expression patterns in peripheral blood that correlate with acute rejection. Using these genes, which encompass multiple biological pathways, a multi-gene test panel was developed that can distinguish quiescence from acute rejection. The test involves real-time PCR expression measurement of a panel of genes derived from peripheral blood cells and applying an algorithm to the results. The algorithm outcome is a single score that considers the contribution of each gene in the panel. This score correlates strongly to immune status and may also be able to predict the occurrence of future rejection and graft dysfunction. XDx Inc expects physicians to combine the algorithm score with other criteria to make clinical decisions. Validation studies of this test and algorithm are ongoing.

Prediction of Rejection to Tailor Anti-Rejection Medications

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 4-year 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 will be studied and this information will be linked to the unique "genomic fingerprint" of a liver transplant candidate, based on the inheritance of more than 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 are no longer 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%.

Role of Immunological Biomarkers in Monitoring Grafted Patients

Gene-expression signatures have been studied in peripheral blood mononuclear cells isolated from patients with autoimmune, graft versus host as well as immunosuppressed transplant recipients. A "sentinel signature" was characterized raising the possibility of application of blood leukocyte expression signatures for assessment of immune status and early detection of disease (Chaussabel et al. 2005).

TcLandscape® technology (TcLand SA) provides both a global and precise picture of T cell mobilization by combining a quantitative and qualitative assessment of TCR gene usage. Originally developed for analysis and monitoring of T cell immune responses, the company now develops specific diagnostic biomarkers as well as a proprietary portfolio of therapeutic molecules for grafted patients. TcLand is currently developing a therapeutic antibody that will selectively inhibit T cell responses directed against the graft. This molecule is a new antagonist of the human CD28 receptor, an important co-stimulatory protein expressed on T cells. Promising preliminary results show that this molecule will be active in the treatment of graft rejection.

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 affects 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.

Personalized Care of Trauma Patients

Traumatic injuries claim hundreds of thousands of lives each year in the USA. 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 inter-individual 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 Anticoagulation

Warfarin is used widely to prevent blood clotting after a heart attack, stroke or major surgery. Anticoagulation by warfarin therapy is complicated by a wide variation among patients in drug response. The oral anticoagulant dose has to be carefully titrated, as too much of the drug can cause excessive bleeding and too little results in no therapeutic benefit. Variants in the gene encoding vitamin K epoxide reductase complex 1 (VKORC1) may affect the response to warfarin. VKORC1 haplotypes can be used to stratify patients into low-, intermediate-, and high-dose warfarin groups and may explain differences in dose requirements among patients

of different racial origins (Rieder et al. 2005). The molecular mechanism of this warfarin dose response appears to be regulated at the transcriptional level. The practical application of this study is that physicians have another tool to personalize anticoagulant therapy. Typically, a patient needs numerous clinic visits before a stabilizing dose of the drug is achieved. This gene test could help them get to the appropriate dose more quickly.

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 2009l). 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 practical currently. 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 O_2 and CMR glu. 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.

Summary

This chapter contains personalized approach to various disorders outside the scope of chapters for cancer, cardiovascular disorders and diseases of the nervous system. 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. The main example is that of HIV infection.

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. Therefore, pharmacogenomics of antiretroviral agents is discussed along with use of molecular diagnostics.

TB is a problem in management due to development of drug resistance. Different genetic mutations may play a role in determining how a patient will respond to the commonly used TB medication isoniazid.

Pharmacogenomic studies on MTX, sulfasalazine and TNF-α inhibitors have been reported, suggesting that the pharmacogenomic approach may be useful for the treatment of RA, a multicomplex system inflammatory disorder for which there is no cure but many therapies are available for relief of pain.

A gene test is now available to personalize anticoagulation therapy with warfarin. 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. SPECT brain imaging is being used to guide hyperbaric oxygen therapy of neurological disorders by identifying responders to this method of treatment.

Chapter 14
Personalized Preventive Medicine

Introduction

The long asymptomatic period before the onset of chronic diseases offers an opportunity for disease prevention. Many chronic diseases may be prevented by avoiding those factors that trigger the disease process (primary prevention) or by use of therapy that modulates the disease process before the onset of clinical symptoms (secondary prevention). Prediction is important for disease prevention so that preemptive treatment can be given to those individuals who are most likely to develop the disease.

Genomics and genetics are vital for the development of preventive medicine. Current practice of preventive healthcare involves general advice applicable to the 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 identify a subpopulation with increased atherosclerosis (Dwyer et al. 2004). The observed diet-gene interactions further suggest that dietary *n*-6 polyunsaturated fatty acids promote, whereas marine *n*-3 fatty acids inhibit, leukotriene-mediated inflammation that leads to atherosclerosis in this subpopulation. These findings could lead to new dietary and targeted molecular approaches to 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

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preventive strategies. A practical scenario in preventive medicine practice could be as follows:

- A patient may need only provide a buccal smear sample in the physician's office for DNA analysis.
- This analysis may eventually be performed for a very reasonable cost.
- This will 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.

Personalized Nutrition

Nutrition plays a crucial role in health as well as disease. 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 and metabolomics in nutrition is already recognized.

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 environment 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 the 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 out diets in accordance with our genetic makeup. A central theme of the research will be to consider whether 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. A project titled "Claims-making in nutrigenomics: A policy-driven analysis of marketing and media" started in 2006 and is due to be completed before end of 2009.

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 counseling.

Nutrigenomics 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. 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 the 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.

Summary

Genomic technologies and genetic screening enable the detection of predisposition to disease before the onset of chronic disease manifestations. Some of the risk factors can be modified and personalized counseling is more effective than general health counseling because the focus is on measures relevant to an individual.

Nutrition is an important part of preventive medicine and nutritional genomics (nutrigenomics) is discussed in this chapter. Nutrigenomics holds the promise to revolutionize both clinical and public health nutrition practice by better targeted nutritional interventions. Personalized diet prescription can be based on genetic variations in individuals.

Chapter 15
Organization of Personalized Medicine

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 15.1. The Personalized Medicine Coalition (PMC) contains many of these players.

Personalized Medicine Coalition

PMC (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 15.2.

The 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 on
  - Personalized medicine: science, policy, and economics
  - Public attitudes toward genetics
  - Personalized medicine and cancer
  - Personalized medicine and psychiatry
  - Public attitudes and trends toward genomics
  - Personalized medicine and reimbursement
  - 'Race' and medicine in the genomics era

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Table 15.1 Players in the development of personalized medicine

Major pharmaceutical companies
Biotechnology companies
Clinical laboratories
Academic sector
Governments
Health insurance carriers
Physicians in practice
Patients

Table 15.2 Members of the PMC

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	Agency Partners
Genomas	Centers for Disease Control and Prevention
Genomic Health	Center for Medicare and Medicaid Services
Genzyme Inc	NCI
IBM Life Sciences Inc	National Human Genome Research Institute
Millennium Pharmaceuticals	FDA
Monogram Biosciences	Academia
Pathway Diagnostics	Duke Univeristy (Durham, NC)
Perlegen Sciences	George Washington University (Washington, DC)
Pfizer	Harvard Medical School-Partners (Boston, MA)
Princeton Group	Healthcare Center for Genetics and Genomics

- 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 a major initiative in the development of personalized medicine. Ten of these companies are profiled in the next chapter. 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 a 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 in which diagnostics and therapeutics are packaged together. The integrated healthcare concept of the company fits in with personalized care. 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. Challenges of drug discovery for personalized medicine are discussed in detail elsewhere (Jain 2006b).

Companies involved in developing personalized medicine belong to several categories: large pharmaceutical companies, molecular diagnostic companies, pharmacogenomic companies, etc. Some are dedicated to developing personalized medicine whereas others have technologies and products that fit in with this system of medicine. Top five companies involved in personalized medicine are shown in Table 15.3.

Table 15.3 Top five companies involved in personalized medicine

Company	Remarks
Hoffmann-La Roche	Largest company in molecular diagnostics as well as a major pharmaceutical company. Pioneer in integrating diagnostics and therapeutics. With acquisition of Ventana, it is the largest personalized medicine company.
GlaxoSmithKline	One of the largest pharmaceutical companies with drug development and clinical trials based on pharmacogenomics and pharmacogenetics.
AstraZeneca	AstraZeneca, a major pharmaceutical company, uses pharmacogenomics and pharmacoproteomics at all stages of drug development.
Perlegen Sciences Inc.	Uses high-density DNA chip sets that make it economically practical for the company's scientists to analyze over 1.7 million SNPs in thousands of individuals to find genetic regions that cause disease or affect drug response. Building its own version of a haplotype map to rapidly compare and analyze whole genomes.
Clinical Data Inc. (CDI)	With acquisition of Genaissance and Icoria, CDI became the premium provider of pharmacogenomic services/biomarker discovery relevant to personalized medicine.

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 book 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 in Chapter 9, manufacturing personalized medicine may become more costly 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 small 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: tested 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 designated as personalized medicine companies whereas others continue to categorize themselves on the basis of 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

BioIT Alliance (http://bioitalliance.org/) 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 plays 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 will help 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.

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 the 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. An example of success of such collaboration is the SNP Consortium (http://snp.cshl.org/), which included 13 companies and five leading academic centers. 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.

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 of 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 the 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 the 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 point of care (POC) 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 POC tests.

Role of the US Government

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 USA.

A bill was introduced in the US Congress in 2006 by Senator Barack Obama (now- President of the United States) titled "Genomics and Personalized Medicine Act of 2006" that aimed to advance personalized medicine and pharmacogenomics. It will be replaced in the upcoming Congress by another bill that includes 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 (HHS).
- 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 by the industrial sector in this domain.

In November 2008, the Department of HHSs released an update of its ongoing efforts in the personalized healthcare arena and the vision that 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 the 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 currently in the USA. It is expected that the current US government, which has shown interest in implementing personalized medicine, will move faster.

Role of US Government Institutions in Development of Personalized Medicine

NIH's Roadmap Initiative for Medical Research

The NIH supports many programs that facilitate the development of personalized medicine although they are not labeled as such. The NIH infused $30 million into its Roadmap initiative in 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 on areas that 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 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, monoclonal antibodies, reagents, growth factors, databases and computer software. All of these have important uses in the 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.

National Institute of General Medical Sciences

In January 2008, the US National Institute of General Medical Sciences of USA (NIGMS) released a strategic plan that outlines its goals over the next 5 years, including the emphasis on continued support for its large-scale research programs such as the Pharmacogenetics Research Network, the National Centers for Systems Biology, the Protein Structure Initiative, and the Models of Infectious Disease Agents Study. 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 portfo-

lio, 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 a 5-year grant of a total of $14.5 million to Duke University.

Other points of emphasis over the following 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 March 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 Pharmacogenomics Knowledge Base (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.

National Institute of Standards and Technology

According to a listing in the Federal Register in December 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 same. 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.

Role of Academic Institutions in the USA

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 non-profit institutes, which has potential applications for personalized care of patients. 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. A few of these programs will be described here briefly.

Clinical Proteomics Program

An example of application of proteomics to the 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.

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. CPMC™ puts the Institute 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 view it 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 which genome information is clinically useful while ensuring that patient privacy is vigorously protected. The study seeks to enroll 10,000 participants by the end of 2009, with an ultimate goal of 100,000 individuals. Coriell is committed to ensuring that the participant population of CPMC™ study 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.

In 2007, 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 world-wide 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 Virtua Health. In March 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.

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 developed in 2004 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. In 2008, EGAPP released a draft of 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. 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 selective serotonin reuptake inhibitors (SSRIs) (EGAPP Recommendation Statement 2007). There was insufficient evidence to support a recommendation for or against the 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%.

Genomic-Based Prospective Medicine Project

In 2003, the Center for the Advancement of Genomics (TCAG) and Duke University Medical Center (DUMC) announced a formal collaboration to create the first fully-integrated, comprehensive practice of genomic-based prospective medicine. Through this new collaboration, Duke and TCAG are generating predictive and prognostic data on specific diseases that can aid both doctors and patients in the earlier detection and better treatment of these illnesses. The activities will include

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 that will arise as a result of advances in genomics. Initially funded internally by both organizations, TCAG and Duke will seek outside funding through government grants, foundations and philanthropic donations. The Duke/TCAG genomic-based medicine collaboration has several initial goals:

1. To integrate high-throughput DNA sequencing technologies and state-of-the-art analysis with distinctive medical expertise by re-sequencing and genotyping the relevant genetic material (genes and regulatory regions) of selected patients from Duke's clinical population. These are patients who have been well characterized through detailed retrospective medical records. By sequencing the DNA of this patient population and associating these profiles with phenotype and disease outcomes, researchers at TCAG and Duke plan to correlate genetic variations to disease states, to be able to initiate preventive steps or earlier treatment of disease.
2. To focus initially on major disease areas, including cardiovascular, hematologic and infectious diseases, as well as cancer. Physicians at Duke's medical center plan to draw up lists of human genes considered likely to play a role in diseases of interest, like the 100 or so genes that may, when mutated, play a role in coronary artery disease. TCAG would sequence the full DNA of these 100 genes from large numbers of patients, looking for the mutations that seemed to be linked to the disease. These mutations could then be used to assess the risk for coronary artery disease in the population at large.
3. To create a futuristic personalized health plan and medical record including genomic information to predict health risks and outcomes from therapy.
4. To leverage the unique high-end computing center that TCAG is currently building as part of its next generation, and the high-throughput DNA sequencing center (the JTC) that is equipped with 100 ABI 3730XL automated DNA sequencers. These will initially enable to sequence 45 billion base pairs of DNA per year. TCAG and Duke along with several technology partners will create unique computing, storage, database and software solutions to manage and mine the massive datasets that will be generated through the genomic medicine collaboration.
5. The collaboration will spur innovation and lower costs of DNA sequencing technologies and TCAG scientists will continue to work toward the goal of a $1,000 genome.

Personalized oncology at Massachusetts General Hospital

In March 2009, oncologists at the Massachusetts General Hospital (MGH), Boston, started to personalize cancer therapy. They plan to read the genetic fingerprints of nearly all the new patients' tumors within a year in a strategy designed to customize treatment. They will search for 110 abnormalities on 13 major cancer genes, that can predict whether drugs already available or in development might be effective against a patient's cancer. High throughput techniques will be used for sequencing 5,000 to 6,000 patients a year, replacing labor-intensive techniques that had been used only selectively for

a handful of cancers. Routine tumor screening has already started with lung cancer, but 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 example of usefulness of this approach was a female non-smoker patient with lung cancer that had not responded to surgery and standard chemotherapy. Genetic screening revealed that the patient's cancer carried the mutation EML4-ALK, which is being targeted by a drug in development, and this patient responded to the drug. 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.

Pharmacogenetics Research Network and Knowledge Base

Pharmacogenetics Research Network and Knowledge Base maintain PharmGKB (http://pharmgkb.org/) at Stanford University (Palo Alto, California). This program is funded by $12 million 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

Quebec Center of Excellence in Personalized Medicine

In February 2008, Montreal Heart Institute and Génome Québec have 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.

Southeast Nebraska Cancer Center's Personalized Medicine Network

In 2005, the Southeast Nebraska Cancer Center (http://www.sncc-of-lincoln.com/) was awarded $1.5 million in US Department of Defense (DoD) appropriations for the current fiscal year to support a network and database of cancer patients' tissue samples. The center is a 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 a "systems biology" approach that brings 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.

Wisconsin Genomics Initiative

In October 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, the University Of Wisconsin School Of Medicine and 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 around 20,000 persons.

Role of Healthcare Organizations and Hospitals

Initially, Healthcare organizations did not show much interest in the implementation of personalized medicine. The first example in the USA is the Signature

Genetics program in Texas. Among the hospitals, the Mayo Clinic is developing a system for personalized medicine and DUMC (Durham, NC) is also involved in personalized medicine. Major health insurance companies such as Blue Cross and Blue Shield are now interested in this topic.

Signature Genetics

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 more than 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.

The Mayo Clinic Genetic Database

The Mayo Clinic (Rochester, MN), in collaboration with the International Business Machines Corporation (IBM), has set up Mayo Clinic Life Sciences System (MCLSS), designed to include detailed genetic information of patients. IBM is digitalizing the genetic profiles in millions of the clinic's patient records. This will help physicians understand how individuals are likely to respond to disease by making it easy to compare them with others of similar genetic profiles and help the development of personalized medicine. Several projects in various therapeutic areas such as management of hypertension and chronic lymphocytic leukemia have already applied a personalized medicine approach. The Mayo Clinic is hoping the database will blend the practice and research of medicine to the benefit of both. In 2007, the AT&T Foundation gave $900,000 to the Mayo Clinic to expand the database of patients' clinical and genomic information. The funds will be used to increase the MCLSS's genomic and prescription data capacities and to make this information retrievable by Mayo Clinic scientists.

Research Center for Personalized Medicine at Mt. Sinai Medical Center

The Mount Sinai Medical Center in New York received a $12.5-million donation from Andrea and Charles Bronfman Philanthropies in 2007 which it will use over 10 years, to start the Charles Bronfman Institute for Personalized Medicine. The research center will study personalized medicine, and the medical center plans to 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 variations and functions 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.

Role of the Medical Profession

Substantial advances that are being made in the area of genomics and the results are beginning to play an important role in the general practice of clinical medicine. The practice of medicine is already being influenced by genomics. It is imperative that physicians involved in clinical practice become more aware of emerging genomic data and participate in integrating medical genomic information into current standard clinical practice.

Education of the Physicians

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 personalized 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 do not have 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 in 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.

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 can

also 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 new course entitled 'Introduction to Pharmacogenomics: Towards Personalized Medicine' in the 2002–2003 academic year. The course is intended for graduate and undergraduate students who have a basic background in pharmacology and in human genetics (Gurwitz et al. 2003).

Education of the Public

Public opinion is an important factor for the implementation of genotyping for pharmacogenetics and pharmacogenomics. There would be several ethical issues arising out of genotyping and detection of genetic diseases. Proper handling of this information will require education of the public about pharmacogenetics and pharmacogenomics. It is anticipated that healthcare companies will play an important role in sponsoring these educational activities.

The individual's right of access to his/her genetic information is well recognized. There is, however, a considerable concern about the application of new genetics approaches. It should be pointed out that application of genetic knowledge is nothing new. Genetic differences in susceptibility to diseases are well recognized in conventional medicine, which is accepted by the public. In public discussions on pharmacogenetics, the scientists and information providers of the industry should avoid getting sidetracked into discussions on the controversial areas of biotechnology.

Role of the Internet in Development of Personalized Medicine

The Internet will play an important role in the development of personalized medicine and the important points are shown in Table 15.4.

An example of the commercial approach to online development of personalized medicine is GeneSage Inc. (www.genesage.com), the first company solely dedicated to developing online solutions to help educate consumers, patients and physicians about the genetic relevance of common and rare medical conditions. This Company has packaged its one-of-a-kind genetic health information system into a new platform that can now be easily and seamlessly adapted for use by healthcare content providers, disease management, pharmaceutical, and clinical testing companies. GeneSage's Rx Platform of detailed information on genetically related conditions,

Table 15.4 Role of the Internet in development of personalized medicine

Education of the public about genetic testing
Information about diseases and early diagnosis for the public
Building of electronic databases and their utilization for research
Internet can reduce the cost and time of drug development
Facilitation of recruitment of patients in clinical trials
Internet would serve as a medium for exchange of ideas about personalized medicine between health professionals

clinical testing and other features was developed to provide a standard framework to respond to the rapidly growing demand for expertise in genomic medicine and health risk assessments. The expert system database, catalogs up to 350 conditions with specific fact sheets covering the most common at two levels: one, designed for consumers or patients, and another for healthcare professionals. The databases can be easily searched by a variety of methods.

Public Attitude Towards Personalized Medicine

It can be anticipated that the public, particularly in the USA, 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 investigating 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, aimed to understand how the development of personalized medicine would 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.(Is the the study yet to begin?or is it ongoing?) According to the NIH, the study will? look into the types of individuals who are and are not interested in receiving genetic testing, what influences their decisions, and how these individuals interact with the health care system. It will?? also aim 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 has 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.

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 in the Developed Countries

Personalized medicine will be initially introduced in the developed countries of the West. The USA is likely to be the first country to introduce personalized medicine on a large scale and some countries in the EU will follow.

US HHSs Supports Personalized Medicine

US Department of HHS is developing an agenda that will put extra emphasis on the development of personalized medicine, and will institute initiatives to ensure that genetic tests are safe and accurate. HHS, which oversees the NIH, has allocated a part of its budget for genetic research into a Genes and Environment Initiative, employing SNP analysis and technology development to understand the causes of common diseases. In addition, HHS has launched a public-private partnership called the Genetic Association Information Network (GAIN) to accelerate genome association studies. Entities in the partnership are NIH, Pfizer, and Affymetrix. The federal funding began in 2007 and will continue for several years. Initial funding was used for genetic analysis. Genotyping studies performed as part of the initiative will be done for several dozen common diseases to be selected by peer review. The genotyping is managed by an NIH coordinating committee under the usual government rules, subject to competition between research facilities. The primary private-sector contributor to the GAIN partnership is Pfizer, which donated $5 million to set up the project's management structure and committed $15 million worth of laboratory studies to determine the genetic contributions to five common diseases. Affymetrix will contribute laboratory resources for two additional diseases, expected to cost about $3 million each. Genotyping supported by Pfizer and donated to the GAIN project will be conducted by Perlagen. A similar arrangement was worked out with Affymetrix. The GAIN initiative proposes to raise $60 million in private

funding for additional genetic studies of common diseases and is actively seeking additional partners. Investigators may submit applications to have genotyping performed on existing DNA samples from patients with specific diseases and control individuals in case–control studies. The National Center for Biotechnology Information at NIH will develop databases to manage the genetic, medical and environmental information resulting from these initiatives. All data will be placed in the public domain.

The HHS already has an Office of Personalized Medicine and an advisory panel that meets regularly to consult and advise on gene-based medical issues. In 2007, HHS funded personalized health care projects with $277 million and the support is proposed to be increased to $352 million in 2008. The HHS initiative has three main goals: (1) to review structures for "ensuring that genetic tests are accurate, valid, and useful by seeing to it that HHS departments know their assignments in this area; (2) by developing consistent policies to guide HHS agencies in managing access to and security of federally supported research; and (3) by creating a network that pulls together health care information from the nation's major health data repositories to "enable researchers to match treatments and outcomes.

Personalized Medicine in the USA

The US healthcare system is undergoing a second wave of change in the beginning of the 21st century similar to the managed care movement in the last decade of the twentieth century. This scenario will be favorable for the development of personalized medicine in which the well-informed public will be a driver. As mentioned earlier in this chapter, some leading clinics and healthcare providers in the USA are already embracing the concept of personalized healthcare.

Personalized Medicine in the EU

There is a tremendous variation in the healthcare systems within the EU but there are some emerging patterns. All EU systems are converging around common denominators that include : more powerful patient organizations, stricter cost control measures, enhanced use of informatics. Patient bodies are a part of the decision-making in most EU systems, even the European Medicines Agency (EMEA), unlike the FDA in the USA. There is an increasing impact of EU regulatory bodies on national healthcare 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. An example will be given of the introduction of genetics on National Health Service in the UK and how it will facilitate the development of personalized medicine.

UK National Health Service and Medical Genetics

In the year 2000, an excellent report from Nuffield Trust in the UK explored the likely effects of genetics on human health and human health services, noting, "the medicine that has been practiced up to now, and the health services that we have become familiar with, will undoubtedly be subject to enormous changes" (Nuffield Trust Genetics Scenario Project 2000). 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 centre 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 £30m ($42m) 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 has nearly doubled to 150 currently. Support staff and genetic counselors have also doubled in number to approximately 500. Research and development in pharmacogenetics is being supported. The number of patients being seen by specialist genetic services has increased by about 80% -to 120,000 a year, and the waiting period to see a specialist has been reduced considerably.

The White Paper generally avoided 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 represented an important milestone in the development of a rational policy for the application of genetic science in healthcare services in the UK. With this background with the organization of the National Health Service in the UK may turn out to be an ideal place to introduce personalized medicine.

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.

Pharmacogenetic data currently available do not comprehensively explain drug response variation within the human populations. One of the many reasons as to why 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 Southeast Asian descent. The results would be available for application to healthcare of nearly 3.5 billion people worldwide who are of Southeast Asian descent. Currently, there is a considerable interest in personalized medicine in Japan, China and South Korea.

Advantages and Limitations of Personalized Medicine

Advantages of personalized medicine for those involved are tabulated as follows: the biopharmaceutical industry (Table 15.5), the patients (Table 15.6), and the physicians (Table 15.7). Limitations of personalized medicine are shown in Table 15.8.

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

Table 15.5 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 the 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 15.6 Advantages of personalized medicine for the patients

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 15.7 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
Increased professional satisfaction

Table 15.8 Limitations of personalized medicine

Factors other than genes also affect response to drugs
Not all the treatments can be personalized
Limited support from governments or healthcare organizations
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

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 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.

Summary

This chapter deals with the organization and development of personalized medicine. Role of academic as well as commercial players in this area is discussed. Healthcare providers as well as the patients are important factors. The US government is facilitating the development of personalized medicine through support of research as well as proposed reforms in the healthcare system. Prospects of personalized medicine in Europe and the rest of the world are described. Finally the advantages as well as drawbacks of personalized medicine are noted.

Chapter 16
Ethical and Regulatory 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, 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 the populations in which they are to be used and the delivery

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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 of 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 seek 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, will be accessible to a wide range of researchers 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 ethical guidance, the research communities should also 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 the freedom to withdraw from research, provide guidance on the t type of information that 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 participating in a whole-genome association studythe 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 rationale 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 (DTC) Genetic Services

Several companies are offering DTC genetic screening tests. DTC advertising for genetic 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. 2005).

A statement released by the American College of Medicine Genetics Board of Directors in 2003 stated: "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 pre-test and post-test 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 concern 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.

Greater regulation is required to oversee the accuracy and quality of "direct to consumer" 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.

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 adequate information to make informed choices about genetic testing is a prerequisite to realizing the public health benefits that have been promised by 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 has asked the Genetics and Public Policy Center (GPPC) at Johns Hopkins University to conduct studies under a $600,000 grant awarded in October 2008. The issues to be studied relate particularly to the ways in which offering genotyping tests and services directly to customers by DTC companies differs from genetic testing offered by healthcare providers. GPPC plans to analyze the current regulations that cover marketing, advertising, and selling of genetic testing directly to consumers, and it will attempt to study 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 will look at how state laws attempting to cover this very new field allow some incoherence and lack of uniformity. The center will also conduct some legal analysis that supports coordinated efforts to protect consumers. The study will not be completed until some time late in 2010.

According to a study by an international team of researchers from the UK, USA, 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.

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. 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 USA

In May 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 reasons 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.

Genotype-Specific Clinical Trials

Genotype-specific clinical trials would include subjects that are 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 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. 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 angiotensin converting enzyme (ACE) inhibitors have little efficacy in blacks.

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 remain 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.

According to other views, 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. The authors of this view believe that 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 low density lipoprotein (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 two to three 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.

In order to address the health concerns of blacks in the USA, Howard University (Washington, DC) started in 2003 to create the nation's largest repository of DNA from African-Americans. The samples would be used to find genes involved in diseases with particularly high rates among blacks like hypertension and diabetes. Howard, a historically black institution, gathered blood samples or cheek swabs from 25,000 people over 5 years, mainly patients at hospitals associated with the Howard College of Medicine. It is expected that genetic information would help to find the causes of disease, predict susceptibility to an illness and choose which drugs would work best for a particular patient.

Regulatory Aspects

The regulatory agencies have not laid down any guidelines for the personalized medicines. Most of the discussions relevant to this topic is covered under the overlapping components of personalized medicine: pharmacogenetics, pharmacogenomics and molecular diagnostics. Regulatory aspects of molecular diagnostics and genetic testing, core technologies for development of personalized medicine, have been discussed in a special report on molecular diagnostics (Jain 2009a).

Accuracy, sensitivity and reproducibility are required for any diagnostic procedure that is to be used for predictive drug testing. In developing genetic test methods, companies should be certified for their testing 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 re-evaluation 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.

CLSI Guideline for the Use of 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 or call 610–688–0100).

Microarray Quality Control Project

The purpose of the MicroArray Quality Control (MAQC) Project is to provide quality control tools to the microarray community in order to avoid procedural failures and to develop guidelines for microarray data analysis by providing the public with large reference datasets along with readily accessible reference RNA samples. MAQC project involves six FDA Centers, major providers of microarray platforms and RNA samples, Environmental Protection Agency, National Institute of Standards and Technology of USA (NIST), academic laboratories, and other stakeholders. The following web site provides further information about MAQC: http://www.fda.gov/nctr/science/centers/toxicoinformatics/maqc/.

MAQC consists of five working groups in its second phase (MAQC-II):

1. The Clinical Working Group, which is analyzing patient data from large-scale clinical studies.
2. The Toxicogenomics Working Group, which is doing the same for toxicogenomics experiments.
3. The Titrations Working Group, which is following up on titration samples from MAQC-I.
4. The Regulatory Biostatistics Working Group, which is advising the Clinical and Toxicogenomics groups on ways to evaluate the performance of predictive models and classifiers.
5. A working group that will identify "best practices" for genome-wide association studies (GWAS).

In phase II, the consortium is addressing the challenges of developing and confirming predictive models that use gene-expression profiles to predict outcomes for individuals, including disease recurrence, prognosis, drug response, etc. However, the increase in the number of array-based GWAS has presented a number of issues that the consortium needs to address as soon as possible. MAQC has successfully proven that microarray technology can be used for biomarker discovery and the group can apply many of the lessons learned from MAQC-I to show that genotyping technology can be just as trusted and just as robust. The FDA has been receiving several GWAS under its Voluntary Exploratory Data Submissions guidelines. These GWAS experiments have enormous sources of variability at each analytical step, yet there has been no framework in the past to explain the rationale of these studies. The goal of the GWAS working group would be to publish best practices for analyzing whole-genome analysis data. The first task for the group, he said, will be to identify experts in academia, industry, and government who will be willing to assist with the project. The ultimate aim is to predict health outcomes based on microarray measurements of biological samples.

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 beginning 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. Unfortunately, the only recent study relevant to this topic focuses on information policy for pharmacogenetics touches superficially on the issues but does not contribute any new or useful information. As personalized medicine gets established, it is expected that the regulatory agencies will work on guidelines for this system. The first step would be the approval of drugs packaged with diagnostic tests.

The GPPC at Johns Hopkins University plans to conduct case studies for cancer, sepsis, and neuroscience drugs and interview officials from the FDA to investigate the regulatory barriers to the development and adoption of pharmacogenetic products. In October 2008, Eli Lilly provided GPPC with 1-year $110,000 grant to study currently regulatory processes for pharmacogenetic drugs and devices. The center anticipates that its findings will aid policy makers in developing policies that will foster pharmacogenetic innovation. The study also will identify impediments to pharmacogenetic innovation and adoption by healthcare providers. It will also propose regulatory reforms. The primary audience for the project is policy makers within the Department of Health and Human Services (HHS) and Congress, and GPPC believes its findings will be useful for the pharmaceutical and medical device industries as well. In October 2008, GPPC also received a $600,000 grant from the National Human Genome Research Institute to study the new DTC genetic testing industry.

Regulation of DTC Genetic Testing

Various states are beginning to tackle the problem of uncontrolled personal genetic services. In April 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 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 June 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 thirteen 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.

FDA and Pharmacogenomics

The FDA issued a document in 2003 – Draft Guidance for Industry: Pharmacogenomic Data Submissions – that encouraged drug and biologic developers to conduct pharmacogenomic tests during drug development and clarified how FDA will evaluate the resulting data. At that time, the FDA Commissioner stated:

"Pharmacogenomics holds great promise to shed scientific light on the often risky and costly process of drug development, and to provide greater confidence about the risks and benefits of drugs in specific populations. Pharmacogenomics is a new field, but we intend to do all we can to use it to promote the development of medicines. By providing practical guidance on how to turn the explosion of pharmacogenomic information into real evidence on new drugs, we are taking an important step toward that goal."

Pharmacogenomics is an area of development the FDA views very positively. The FDA received more than 20 drug submissions that included pharmacogenomic data within a year of issuing guidance on how to do so in 2005. Two cancer drugs were approved that include this type of 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 2005. Current information relevant to pharmacogenomics is available on FDA's website (http://www.fda.gov/cber/gdlns/pharmdtasub.htm). 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 the data that 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 will not be used for regulatory decision making;it will however 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 phamacogenomics 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 side effects of drugs.

The guidance provides specific criteria and recommendations for submission of pharmacogenomic data investigational new drug (INDs) and new drug application (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; he agency assures 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 the 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 USA 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 Information in Drug Labels

Currently, the FDA wants to see genomic information on the front and center in a drug's label. The agency is poised to release guidelines for the "Clinical Pharmacology

Section of Labeling for New Prescription Drugs, Content and Format". The format for new drug labels will include 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.

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 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

This FDA guidance also makes a distinction between pharmacogenomic tests that may be considered as probable or known valid biomarkers, or 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 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, toxicologic, 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 the tests that 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, FDA will continue to seek public comment as it evaluates whether a biomarker is a valid biomarker (e.g., via discussions at Advisory Committee meetings) as and when appropriate.

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 draw 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 co-operation 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 (Lesko and Woodcock 2004).

FDA Regulation of Multivariate Index Assays

In 2006, the FDA took a step towards 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 control or supervision of 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 MIAs'' in 2007 and comments were invited. As of March 2009, no decision had been made by the FDA. Pharmacogenomic information is contained in the label of approximately 10% of all FDA-approved drugs. Included among those are trastuzumab for breast cancer, 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 home-brew tests, have been generally considered as laboratory services 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 important 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, in a change of its policy described earlier in this chapter under the heading "Regulation of IVD by the FDA", 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 as a prototype guidance that will provide a general framework for how the FDA's Office of In Vitro Diagnostics approach IVD MIAs. The FDA cleared the first such IVDMIA device – Agendia's MammaPrint test – in 2007, which it had originally classified as a Class III device that would have required full premarket approval. However, Agendia had filed a petition requesting that the device be reclassified into Class II, which only requires 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 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 realtime 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 been cleared as Class II devices.

Evaluation of Companion Diagnostics/Therapeutic for Cancer

Currently, there is no proven development pathway for FDA approval of the necessary companion diagnostic tests and their associated targeted therapies. In 2007, the Critical Path Institute (Tempe, AZ) announced that it will use 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. Ventana Medical Systems will test the resulting process. The goal of this collaboration is to establish the performance standards that would serve as the model for future FDA co-submissions of companion diagnostic tests and cancer drugs. The first test to which the groups plan to apply the standards will be a diagnostic for lung cancer produced by Ventana. The ultimate goal of the project is to guide the choice of targeted therapy so that patients receive the most effective treatments.

Summary

This chapter discusses the ethical issues of personalized medicine, which mainly pertain to genetic information and protection of privacy issues. The Genetic Information Non-disclosure Act in the USA protects against any misuse of genetic information.

There are no serious regulatory issues involved in the development of personalized medicine. Molecular diagnostic tests used in the practice of personalized medicine as well as companion diagnostics for drugs require regulatory approval. Validation of biomarkers is important for their? use in personalized medicine. The FDA encourages the submission of pharmacogenetic and pharmacogenomic information during development but it is not mandatory.

Chapter 17
Economics of Personalized Medicine

Introduction

The 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 will be discussed later in this chapter. A more detailed description of the commercial aspects of personalized medicine including markets and profiles of companies involved in developing various relevant technologies can be found in a special report on this topic (Jain 2009g).

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 over $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 the part of the pharmaceutical industry.

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Personalized Medicine and Orphan Drug Syndrome

An orphan disease is a condition that affects less than 1 person per 100,000 population. 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 USA and similar laws in the European Union, Japan, and some other countries provide financial incentives for the pharmaceutical companies developing products for orphan diseases. Potential problems in this area 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 developing such medicines. Systematic pharmacoeconomic studies of pharmacogenomics have not yet been carried out. The economic benefits can be predicted on the basis of the current progress made in genomics and will be a sequel to reduced time for R&D and introduction of the product into the market.

Cost of DNA Testing

DNA tests for identifying an individual are 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 2009a).

Cost of Sequencing the Human Genome

Currently it is very expensive to sequence the 3 billion base pairs of DNA found in humans. Therefore, large scale sequencing is 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 to a 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 by 2015 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. A survey of the new approaches in development for reading the genome indicates the potential for breakthroughs that could produce a $5,000 human genome by 2009 and $1,000 genome before 2015.

In 2007, NHGRI pumped over $15 million into twelve 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. The current round of next-generation sequencing grants were awarded to eight researchers who are working on developing technology to enable the $1,000 genome, and to three scientists who will try to develop sequencing technology that will sequence the human genome for $100,000 or less. The different approaches will likely result in several successful and complementary technologies and NHGRI will monitor carefully to see how each technology progresses and which of them can ultimately be used by the average researcher or health care provider. Recipients of the NHGRI $1,000 Genome grants were:

1. Duke University's project "Continuous Sequencing-by-Synthesis, Based on a Digital Microfluidic Platform." The team uses droplet-based microfluidics in sequencing-by-synthesis studies aimed at extending read length, minimizing reaction volume and increasing throughput to 10,000 reactions in a very small area.
2. Arizona State University's project "Sequencing by Recognition." This research team seeks to develop molecular wires that are sufficiently flexible and sensitive to allow for use in 'sequencing by recognition' methods involving nanopores.
3. Brown University's "Hybridization-Assisted Nanopore DNA Sequencing." This group uses solid-state nanopores to find where DNA sequences attach by hybridization, which through repetition may allow determination of long strands of DNA.
4. University of Medicine and Dentistry of New Jersey's "Ribosome-Based Single Molecule Method to Acquire Sequence Data from Genomes." The group has modified key ribosome components to read nucleotide sequences. The group anticipates that DNA sequences could be determined by sequencing mRNA.
5. University of British Columbia (Vancouver, Canada) "Nanopore Array Force Spectroscopy Chip for Rapid Clinical Genotyping." This team is developing solid-state, nanopore-based force spectroscopy to detect sequence variation. The team previously demonstrated the ability to detect sequences "at single base resolution using organic nanopore force spectroscopy."
6. NABsys' "Hybridization-Assisted Nanopore Sequencing." The company is working with a group at Brown University to develop biochemical and algorithmic components for a sequencing-by-hybridization method.
7. North Carolina State University's "Sequencing DNA by Transverse Electrical Measurements in Nanochannels." The team aims to stretch long DNA molecules

by passing them through nanofluidic channels, then to fit nanoelectrodes into those channels to detect electrical signal of DNA bases.
8. University of California at Irvine's "High-Throughput, Low-Cost DNA Sequencing Using Probe Tip Arrays." The group is using nanoscale electrophoretic separation of DNA fragments on an atomic force microscope probe tip in an effort to speed up and scale down the Sanger sequencing method. Then it will implement these "very challenging separations" on a massively parallel sequencing platform that contains hundreds of probe tips.

Recipients of the NHGRI $100,000 Genome grants are:

1. University of New Mexico School of Medicine's "Polony Sequencing the Human Genome." This group's goal is to use polony genome sequencing technology to resequence the human genome "within a week for less than $10,000" by improving sequencing data and advancing the computational tools that are used in genome assembly.
2. Columbia University has two grants. In the first, "3′-*O*-Modified Nucleotide Reversible Terminators for Pyrosequencing." the researcher is using the funds to design a library of synthetic molecular tools intended to optimize pyrosequencing.
3. In Columbia University's second grant, "An Integrated System for DNA Sequencing by Synthesis," the group is continuing to develop and optimize a set of fluorescent nucleotide reversible terminators for sequencing-by-synthesis, and it will work to develop a new method for prepping DNA beads for attachment to a substrate.
4. University of Wisconsin, Madison "Sequence Acquisition from Mapped Single DNA Molecules." This team is developing a system for analyzing large amounts of human genome data that connects the location of sequence elements to map information, and will include information about structural variations and aberrations that could be linked to other sequencing data.

In 2006, Genome X Prize Foundation of New York announced a $10 million cash prize for the first team to develop a device that can sequence 100 diploid human genomes in 10 days for $1 million ($10,000 per genome). At present, the bar for data quality is set very high: Sequence data submitted must cover 98% of each genome with no more than one error per 10,000 base pairs. Several industry players have already entered the quest, including VisiGen Biotechnologies, 454 Life Sciences (a subsidiary of Roche Biosciences), and the Foundation for Applied Molecular Evolution. In May 2007, Reveo joined the race with Omni Molecular Recognizer Application, which uses principles from semiconductor electronics and photonics rather than indirect chemical methods to read the DNA sequence directly. The company plans to use arrays of nano-knife-edge probes to directly and nondestructively read the human gene sequence. In May 2007, 454 Life Sciences sequenced the first individual human genome for less than $1 million.

In 2007, BioNanomatrix and Complete Genomics formed a joint venture to share a grant from the US National Institute of Standards and Technology to develop

technology that will be able to sequence a human genome in 8 h for less than $100. The proposed sequencing platform will use Complete Genomics Inc.'s sequencing chemistry and BioNanomatrix' nanofluidic technology. The companies plan to adapt DNA sequencing chemistry with linearized nanoscale DNA imaging to create a system that can read DNA sequences longer than 100,000 bases quickly and with accuracy exceeding the current industry standard.

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 2008, the cost is about $100,000. Knome, a company that offers to provide consumers with their DNA sequence, charges $350,000 that includes not just the sequencing costs but also the analysis of the data and the customer service. Life Technologies expects that its newest machine would allow a human genome to be sequenced for $10,000, although that includes only the cost of consumable materials, not labor or the machinery. Thus, the cost of DNA sequencing dropped by a factor of 10 every year from 2004 to 2008.

Complete Genomics start will be 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 does not work that much differently from rival machines, but miniaturization enables it to use only tiny amounts of enzymes and other materials. Such a price would represent another step toward the long-sought goal of the "$1,000 genome." At that price point it might become commonplace 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 will not offer service to consumers, but it will provide sequencing for consumer-oriented companies like Knome. Most of its customers are expected to be pharmaceutical companies or research laboratories that are conducting 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 expects to perform 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.

VisiGen Biotechnologies Inc was awarded a "$1,000 Genome" grant from NHGRI to advance the development of innovative sequencing technologies intended to reduce the cost of DNA sequencing.

Cost of Genotyping

Currently, it typically costs a drug company about $800 million 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 will 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 methodology 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 and 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 utilized widely the cost per genotype has to come down from the current cost to 0.1¢ per genotype. Current genotyping arrays can tell us 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 when 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. Some companies are now using genotyping in most of their trials while others are not.

Cost of Personalized Healthcare

Cost of Genetic Testing

The cost/effectiveness of pharmacogenetic testing has not been studied extensively. Although there would be added costs of genotyping, considerable unnecessary expenses can be saved in drug development. In medical practice, the cost associated with screening all individuals before drug administration can be offset by a reduction in costs associated with adverse reactions and therapeutic failures. The current empirical method of drug prescription where a doctor tries a drug and tells the patient that it may work and if it does not, he will switch to another. This trial and error method is not only expensive but also harmful for the patient. Personalized medicines, which are tailored to a patient's needs and selected on a genomic basis, are going to be definitely effective and safer. Therefore, there should be significant long-term cost savings for the healthcare sector in a managed care environment. An additional benefit of combining diagnostics with therapeutics would be preventive medical treatment as required to prevent the full-blown disease, which would cost more to treat. This is the concept of "Predictive Medicine" approach.

A closely watched test in this segment is AmpliChip (Roche) for pharmacogenetics, which has not been adopted as widely as expected even though its benefits have been proven. Roche sells AmpliChip for $400 but the test is offered by only four clinical reference laboratories, which charge between $600 and $1,200 for the test with no evidence that it is regularly reimbursed by insurance companies.

In 2006, Mayo Collaborative Services and Medco, a Pharmacy benefits management company, announced their plan to study whether using genetic tests can cut costs and improve care for patients taking the anticoagulant warfarin. They will evaluate test data from more than 1,000 subjects out of the roughly 200,000 individuals Medco tracks every year and who are new to warfarin treatment. The genetic testing will be performed by the Mayo Clinic's department of laboratory medicine and pathology, and the results will be forwarded to physicians to help them determine correct drug dosage. The study will follow patients through the normal course of treatment and will be completed at the end of 2007. The study is the first in a line of similar collaborations that will explore the financial and health benefits of genetic tests used with other drugs. This information will be important for assessing the cost effectiveness of personalized medicine.

The cost of direct-to-consumer personal genetic testing varies among the various companies. The cost of 23andMe's service, at $399, is the least expensive. Navigenics' SNP-genotyping service, which uses Affymetrix arrays, costs $2,500, while Decode Genetics' program, which uses Illumina's Human 1M BeadChips, runs $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 between 12 and 25 SNPs linked to certain conditions. Meantime, a full-genome scan by Knome costs at least $350,000.

Economics of CYP Genotyping-Based Pharmacotherapy

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 nonfunctional alleles, phenotypically characterized as "poor metabolizers" (1–10% of Caucasians). Pharmacogenetic analyses can significantly contribute to reducing treatment costs for ADRs 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) may be less than the cost of lost work and wages due to ADRs. The pharmacogenetic analyses are coming to a point where they may drive down costs incurred by illness.

Cost of Personalized Medicines

Overall, health care inflation continues to rise precipitously. In 2000, total health care expenditures in the USA were $1.3 trillion and they doubled in 2005 and are continuing to increase since then. Hospital care, physician services, and prescription drugs accounted for most of this total spending. In spite of rising costs, the quality of care has declined. The health care system in the USA is in need of a new paradigm to change this inflation rate. Personalized preventive or prospective medicine may improve as well as lower 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 nonsurgical therapies such as gene therapy would be developed.

Currently less than 15% of the world's healthcare budget is spent on drugs. It is likely to increase during the next decade, depending upon the kind of new and effective medicines that come out of the pipeline. Many of the currently incurable diseases such as Alzheimer's disease will have rational therapies. The introduction of treatments for incurable diseases would raise the drug costs but 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. An example is given in the following section.

Lowering the Cost of Healthcare in the USA

No field study has been done so far to determine the 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 the costs due to adverse reactions to conventional drugs.
- Reduction of the high expense of hospital stay.
- Predictive medicine will reduce costs by prevention.

Cost Effectiveness of HIV Genotyping

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 is due to HIV drug resistance and a subsequent shift to more complex and costly therapies. Genotypic guided treatment is associated with better virologic outcome. Several studies have shown that genotypic for antiretroviral resistance 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.

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 markers will enable us to decide who really needs expensive therapy. The costs will be reduced significantly as more genetic variants that are most important in terms of drug response come into play. 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. Nonresponders to a particular chemotherapy could be spared unnecessary exposure and adverse effects.

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 Centre d'Etude du Polymorphisme Humain (CEPH, 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. Researchers 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.

Reducing the Cost Incurred by Adverse Drug Reactions

Over 2 million adverse drug reactions that occur in the USA per year cost approximately $25 billion. 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.

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.

Overall Impact of Personalized Medicine on Healthcare

Increase in the treatment efficacy of individualized treatment is difficult to measure in financial terms but the savings from reduction of adverse reactions would be considerable. Adverse reactions to medicines in hospitalized patients in the USA, or admissions to hospital because of an adverse event are estimated to cost over $100 billions per year to the healthcare industry. Even if personalized medicine reduces adverse reactions by a small percentage, the resulting savings to the healthcare industry would be considerable.

Summary

Initially the pharmaceutical industry was concerned that developing personalized medicines would be less profitable than the conventional pharmaceuticals. The concepts and models for personalized medicine are now accepted by the

pharmaceutical industry and the biotechnology companies involved in this area have better business prospects. The next question is if the healthcare providers can afford the perceived higher cost of personalized medicine. Information presented in this chapter shows that although the initial cost of testing may be high, personalized medicine will reduce healthcare costs by eliminating the wasteful use of ineffective drugs and reduce the cost of care for adverse effects of drugs. Current efforts are directed at reducing the cost of DNA sequencing and $100 genome should be a reality within the next few years.

Chapter 18
Future of Personalized Medicine

Introduction

Based on the current progress in biotechnology and molecular medicine, considerable advances and breakthroughs are expected in the second decade of the twenty-first century. These will involve progress towards finding a cure for cancer and making it a chronic manageable disease. Many of the advances will be through application of new technologies such as nanobiotechnology and refinements of cell therapy, particularly the use of stem cells. Automation, robotics and informatics will be partially integrated into clinical medicine. Advances in regenerative medicine and tissue engineering will enable repair and regeneration of damage in CNS and cardiac disorders. The emphasis in treatment of neurological disorders will be on neuroprotection rather than control of symptoms. Management of infectious diseases will improve although unpredictable challenges may arise from emerging viral infections.

In the setting of this progress, personalized medicine will be an important part of managing patients. Advances in molecular diagnostics and discovery of biomarkers will facilitate this development. Important advances relevant to personalized medicine will be:

- 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.
- 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.
- Preventive medicine will be well recognized with acceptance of presymptomatic diagnosis and pre-emptive treatments.

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Ongoing Genomic Projects

Several studies of the human genome are still going on and some are planned. A selection is described briefly in the following pages.

Understanding the Genetic Basis of Diseases

Although molecular diagnostics has already made considerable advances, the technologies have not been applied to understanding the genetic basis of disease, which is important for developing personalized medicine. To some extent, it is due to lack of funding for research projects investigating the genetic basis of diseases. Some of the ethical and social issues of genetic screening also need to be resolved. One example of a project to investigate genetic basis of breast cancer is a pioneer step in this direction.

Since 2005, the University of Cambridge (UK), Cancer Research Technology, Cancer Research UK and Perlegen Sciences Inc are conducting a collaborative high-resolution, whole genome association study on breast cancer. Scientists will determine over 200 million individual genotypes in DNA samples collected from patients to further elucidate the genetic basis of the disease. As the most comprehensive search ever conducted into the genetic basis of breast cancer, this project may help to identify, more precisely, women at high risk of the disease, and may ultimately lead to improvements in the prevention, earlier detection and treatment of breast cancer. The study will be a genome-wide scan for common predisposing genetic variants that are associated with susceptibility to breast cancer. Genetic variants in genes such as BRCA1 and BRCA2, which predispose strongly to breast cancer, have been identified previously, but these are quite rare and account for less than 5% of breast cancer cases. This new study hopes to provide a more comprehensive understanding of the genetic basis of breast cancer.

Personal Genome Project

Low cost personal genome data is important for the implementation of personalized medicine on a large scale. A Personal Genome Project (PGP) has been launched as a sequel of the Human Genome project and volunteers are being recruited to make their own genomic and phenomic data available (Church 2006). These resources will include full (46-chromosome) genome sequences, digital medical records and other medical information that would become a part of personal health profile. It will include comprehensive data about RNA and protein, body and facial measurements and imaging such as MRI. Human cell lines representing each subject will be deposited in a repository at the National Institute of Genome Medical Sciences. The subjects will sign an informed consent and although the subjects will be fully identified, the privacy of the individual will be respected and the data

will be protected from hackers. Details of PGP can be found at the following web site: http://arep.med.harvard.edu/PGP/. According to this web site, the project is intended to stimulate a "critical mass of interested users, tools for obtaining and interpreting genome information, and supportive policy, research, and service communities." So far ten individuals, including Dr. Church, have enrolled. In time, organizers hope to enroll 100,000 participants. Personal Genomes Organization is committed to making research data from the PGP freely available to the public.

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. 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 GWASs. NIH is seeking applicants who will "develop and test innovative, informative, and cost-effective methods and analytical strategies for identifying gene-environment interactions in

GWASs, 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

It was announced in 2008 that the 1000 Genomes Project will be 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. The collaborators expect to finish sequencing 1,200 human genomes by the end of 2009. Meanwhile, the three 1000 Genomes pilot projects, which began in 2008 and are aimed at achieving 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 and is expected to increase that dramatically, producing a petabyte of data.

Beyond the direct implications of 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 is 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 Parsis in India lives beyond the age of 60, compared to the national average of 7% of survival beyond 60 in the whole population of India (http://www.unescoparzor.com/). 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 2008, the team had performed expression profiling and transcript mapping experiments across a subset of the samples. The project is expected to be completed before 2013. All of the 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 medicine deals with transfer of technologies from preclinical research into clinical application. Methods of translational medicine that are relevant to personalized medicine are shown in Table 18.1. Biomarkers play an important role and this has been discussed earlier in the report.

Translation of Genomic Research into Genetic Testing for Healthcare

Advances in genomics have led to mounting expectations with 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 was 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:

Table 18.1 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

- 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, Affymetrix, and Microsoft embarked on a decade-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 the diseases and the application of this genetic information for preventing, diagnosing, and treating diseases. Affymetrix will perform the genome scans, while Navigenics will interpret the results and offer guidance on steps individuals can take to try to decrease health risks based on their personal genetic information.

Drivers for the Development of Personalized Medicine

Various drivers for the development of personalized medicine in the next decade are listed in Table 18.2.

Table 18.2 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

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. 18.1 will act as drivers for the markets.

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. Predictive medicine involves prediction of risk of disease in an individual and its personalized management. It is sometimes referred to as

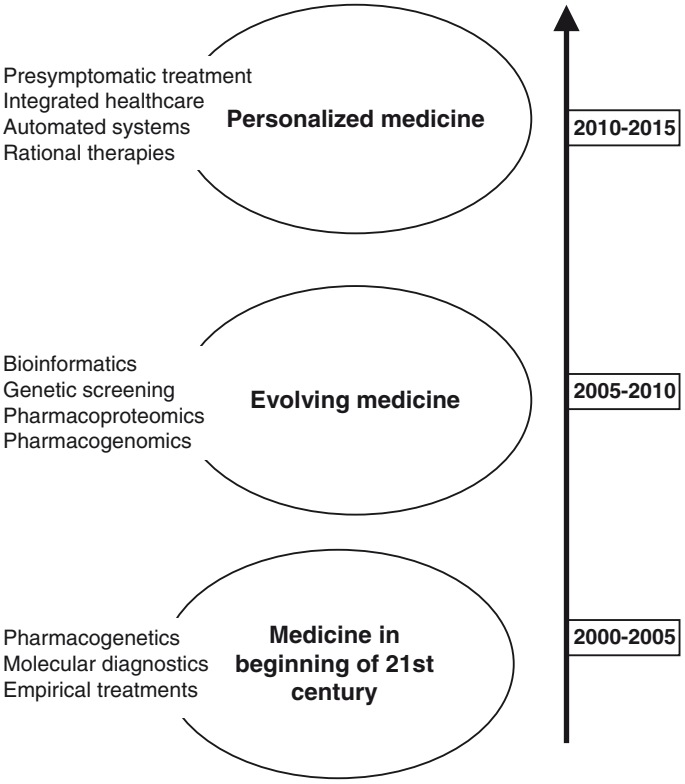

Fig. 18.1 Evolution of personalized medicine as a market driver. © Jain PharmaBiotech

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 of cure of diseases such as 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 care. 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.

Opportunities and Challenges

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 2009). Thus, schizophrenia would be caused by combinations of 1,000 rare genetic variants, not of 10 common genetic variants. However, deCODE Genetics, which also offers a personal genome testing service, alerts clients to pay attention to diseases for which testing shows their risk is three times as great as average, but not for trivial increases in risk. According to deCode scientists the undiscovered share of genetic risk for common diseases probably lies not with rare variants, but in unexpected biological mechanisms. DeCODE has found, e.g., that the same genetic variant carries risks that differ depending on whether it is inherited from the mother or the father.

According to another expert opinion, which disagrees with skeptics, genomewide association studies will have yielded important new biologic insights for at least four common diseases or polygenic traits by 2012 and that efforts to develop new and improved treatments and preventive measures on the basis of these insights will be well under way (Hirschhorn 2009). 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. The situation may be very different by 2012. 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 (Kraft and Hunter 2009).

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.

Challenges in Delivery of Personalized Medicine

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 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 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 to anticipating and decreasing risk for 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.

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. The recent advances in silico modeling for predicting the absorption, distribution, metabolism, and excretion (ADME) could be incorporated into this system.

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 2010. Although some of the pharmacogenomics-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 markers is already in use. Integration of diagnostics and therapeutics is also taking place and it is anticipated that personalized medicine will develop parallelly with the introduction of pharmacogenomic-based medicines.

Concluding Remarks about the Future of Personalized Medicine

Going back to the year 1998, when the first edition of this report was published, there was little interest in personalized medicine. Currently, there is a tremendous interest in this topic, but there are still many misconceptions about the scope of personalized medicine. Some 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 the 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 has been disputed (Jain 2006a). Even though the Royal Society claims to have consulted a broad spectrum of persons and organizations involved in personalized medicine, they took scant evidence from the most important players – the biopharmaceutical industry. 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 fulfill 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.

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 USA by the year 2013 and in the UK by the year 2015. 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 preeminent position of research activity in genomic medicine, has good prospects for introduction of personalized medicine.

Summary

In the setting of anticipated progress in healthcare in the second decade of the twenty-first century, personalized medicine will be an important part of managing patients. The ongoing projects will improve our understanding of the disease as a basis for personalized medicine. Various drivers for the development of personalized medicine, both scientific and socioeconomic, have been identified. Controversies about the value of genetic information in predicting disease are being resolved. Overall there are good prospects for wider acceptance of personalized medicine by the year 2013 in the USA.

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