Chapter 19

Genomics, New Drug Development, and Precision Medicines

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Chapter Outline

INTRODUCTION

Pharmaceutical companies have historically focused their drug discovery and development programs on finding therapies for broad use in large disease populations, the so-called "blockbuster business model." A blockbuster drug is usually defined as one with peak annual sales of greater than $1 billion and is generally developed for long-term use to treat common complex chronic disorders in the general population. The strategy to identify and develop blockbuster drugs has been the response to the high cost of drug discovery and development. A survey of the drug development costs of 68 new compounds from 10 pharmaceutical companies estimated that the cost to develop a new drug in 2000 was $802 million [1]. The high cost of developing drugs can be attributed to two main factors: the large size and duration of the clinical trials required to provide the data to show safety and efficacy of the compound, and the high rate of attrition of compounds in clinical development. Fewer than 10% of compounds entering phase I clinical development reach the market, the majority failing in clinical development because lack of efficacy in phase II. This lack of recent research and development success in finding blockbuster drugs combined with financial pressure caused by patent expiration and downward pressure on pricing has led to a shift in strategy for many companies within the biopharmaceutical industry. Companies are shifting toward the discovery and development of stratified medicines. A stratified medicine is one that is targeted at a subgroup of a traditionally classified disease; eg, trastuzumab (Herceptin) for the treatment of *Her2* overexpressing breast cancer. Stratified medicines offer significant opportunity to the industry because they have an increased probability of success and the potential of smaller programs, to the regulators as the benefit-risk profiles of these medications are greater than unselected medications, to the payers because they are more cost-effective, and most importantly to patients because they are more effective and safer therapies. Genomics has a large role to play in the development of stratified medicines because many of the tools used to stratify the patient populations are genomic; eg, epidermal growth factor receptor (*EGFR*) mutation status and gefitinib, *KRAS* mutation status and cetuximab (Erbitux) and panitumumab (Vectibix), *ALK4* mutation status and crizotinib.

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Pharmacogenomics, the investigation of variations of DNA and RNA characteristics (germline or tumor) as related to drug response in individual patients or groups of patients, is one of a number of initiatives employed by the pharmaceutical industry to stratify patient populations.

A major cause of the attrition of drugs for lack of efficacy is the heterogeneity of the diseases we currently classify as single entities. Most would be better referred to as *syndromes* rather than single diseases. The disease classification currently used is based on phenotypic consequences of disease processes rather than the underlying pathological mechanisms. This has led to the clustering of heterogeneous disease syndromes based on symptoms rather than based on molecular pathology. Genomics will be an important tool in reclassifying diseases into a new molecular taxonomy of human disease. Oncology is one therapeutic area where this is most advanced because the scientific evidence base for tumor etiology is more advanced than in other area. The majority of drug development programs in oncology are now stratifying patient populations based on molecular changes in the tumor. It is widely expected that this approach will expand across other therapeutic areas as our understanding of disease biology improves.

The Drug Discovery and Development Process

The generation of an idea that a particular protein might be a suitable therapeutic target for the treatment of a disease sets in motion what is often depicted as a linear process known as the drug discovery and development pipeline, in which new medicines follow a set route from early discovery and preclinical stages through a set of clinical development processes to the marketplace. In reality the process is generally far from linear, but for the purposes of describing the component parts we will consider it a sequential process.

The ultimate aim of the drug discovery process is to find a chemical (eg, small molecule) or biological reagent, such as an antibody, which has the potential to be a drug that can be moved into preclinical and then clinical testing. In order to start the process of identifying a potential drug, a biological assay testing interactions with the drug target must be developed. This assay is generally based on a cloned and expressed form of the drug target and will be converted into a format that will allow high-throughput testing, as millions of chemicals may need to be screened in the assay. The need to screen millions of chemicals means that it is usually only feasible to screen one protein variant of the target in the high-throughput screen. It is therefore vital to screen the "right" variant. In the situation where there may be more than one form of the protein that can be included in the screen, it is important to know that the most biologically relevant and/or the most common variant is screened, and it may be necessary to screen the chemical matter against more than one form of the protein. This is not always the most common form of the protein; for example, verumafenib, a novel drug for the treatment of malignant melanoma, was identified by specifically screening against the V600E mutated form of the *BRAF* protein to ensure it only blocked signaling of the pathogenic form.

The high-throughput screens generally identify several potential "hits," which need to be tested in more rigorous biological assays to determine the type of interaction and the effects. Promising "leads" are then developed by a series of minor chemical changes to the original lead, and the final candidate is chosen based on the selectivity and potency criteria required for the drug candidate. This candidate is then taken forward into preclinical testing.

The final testing phase is usually based on in vivo testing of the compound in animal models that are demonstrated to have some translatability to the target human disease or a range of ex vivo models of human tissue recapitulating components of the disease. The predictability and translatability of these models to humans varies with different diseases and is the focus of biomedical research in many therapeutic areas.

Preclinical Testing

Once a drug candidate has been made, it goes into a range of preclinical toxicology testing that includes in vitro screening tests to identify potential pharmacological effects at other receptors that could lead to adverse events, and genetic toxicology testing, which evaluates mutagenicity and pathogenicity. Only if these are satisfactory does animal testing begin. The animal testing is done in two species and is staged to ensure that as few animals as possible are used and that major problems are picked up early. Toxicology studies to evaluate long-term exposure, reproductive toxicological effects, juvenile toxicity, and carcinogenicity are generally only performed once the data have been obtained from short-term human studies that support safety and efficacy. To date, toxicology induced by new chemicals is identified and classified by standard phenotypic and histological changes. Although this picks up the majority of potential toxic effects, it can be insensitive to subtle changes and can identify species-specific effects that can be difficult to interpret. A greater understanding of the molecular changes after drug administration could identify more subtle effects and species-specific effects. The applicability of animal models of disease could also be assessed by evaluating molecular changes rather than probably misleading phenotypic similarities. Adverse events can be caused by unexpected consequences of the primary pharmacology or by unexpected interactions

with off-target proteins. Understanding the mechanism of the toxicological effects is important, because this allows a more quantitative evaluation of the risk of the event happening in humans. Genomics can be used to identify interactions with off-target proteins, because transcription changes induced in the organ damaged by the compound can point to the mechanism of the toxicity. This is often referred to as *toxicogenomics*. Multiple consortia, eg, the Predictive Safety Testing Consortium and SafeSciMET, are working to identify genomic biomarkers that are more sensitive than current histopathological scores, allowing early detection of toxicology and the demonstration of species-specific toxic effects. Similarly where specific organ toxicity is expected because of the mechanism of action of the compound or known off-target effects, then transcription changes can offer a more sensitive assay to detect early organ damage.

Clinical Development

Once the initial in vitro testing and acute animal toxicology studies (generally 14 days) have been performed, then it is possible to start testing the candidate drug in humans. The human studies have traditionally been split into four phases (I–IV), each with specific aims (Box 19.1).

Phase I

The first time a novel compound (or biological therapy) is tested in humans, a broad range of dosages and dosing strategies are tested starting at very low exposures to minimize any risks to the clinical trial participants. Although these initial studies have generally been performed on healthy volunteers, there is an increasing trend toward incorporating patients as early as possible. The dose is escalated over several weeks starting at between 10- and 100-fold below the expected pharmacological exposure levels to a maximum tolerable level or several-fold beyond the expected maximum clinical dosage (whichever is reached sooner). The aim is to identify common adverse events and the relationship with plasma exposure as well as to establish the basic pharmacokinetic (PK) parameters. As drug development continues, more studies are performed to understand the effects of multiple dosing, specific drug–drug interactions, and food effects. The aim of these studies is to provide a more comprehensive understanding of the PKs and significant causes of variability in PK profiles. Collections of pharmacogenomic samples in phase I clinical protocols allow the assessment of the impact of genetic variation on drug metabolism and transport.

There is a growing trend for performing some of these very early studies in patients and these are often referred to as *phase Ib studies*. The primary intent of these studies is still to establish safety and PKs of the compound, but the use of patients allows early indicators of target engagement and biomarkers of efficacy to provide evidence that the compound is modulating the proposed mechanism. Where it is possible to biopsy disease tissue in these studies, then white cell transcription analysis can provide some evidence that the target pathway is being modulated. This is generally restricted to some tumor types and dermatological conditions; eg, psoriasis where it is possible to obtain high-quality tissue samples.

Phase II

Phase II is traditionally divided into phase IIa, where the aim is to demonstrate the safety and PK parameters in patients, and IIb, where the aim is to establish efficacy and delineate the dose–response curve. However, most companies now endeavor to generate some biomarker data in the phase IIa studies to provide some evidence of efficacy and confidence to progress into the more expensive and larger phase IIb dose ranging study. This is a critical time, because up to 50% of all drug candidates will fail in phase II. If preclinical data or data from translational medicine studies have identified a patient population more likely to respond to the mechanism, eg, *BRAF* activating mutation-positive melanoma tumors for MEK inhibitors, then the studies can be restricted to this patient population to increase the likelihood of seeing an efficacy signal. Even when there is no strong a priori hypothesis then samples collected in phase II studies for pharmacogenomic analysis are useful for testing less validated hypotheses on the impact of genetic variation with respect to drug response, particularly for genes with large effects, because these studies are limited in that they comprise relatively small numbers of patients (50–100). Samples for these pharmacogenomic studies may be collected with specific consent for genotyping of named genes within the protocol, which can be correlated with clinical data collected in the trial.

Box 19.1 Human Studies Have Traditionally Been Split into Four Phases (Phases I–IV)

- Phase I: pharmacokinetic (PK) and safety profiles in healthy volunteers
- Phase II: safety and efficacy in patients and the establishment of the dose response
- Phase III: safety and efficacy at the chosen dosage
- Phase IV: postapproval studies to answer specific safety or efficacy questions and to support commercial strategies

Phase III

Phase III trials form the basis of the regulatory approval, and they are large studies evaluating the safety and efficacy of the candidate at the clinical dose and in the population where the drug will ultimately be used. The cost of this phase of development is significantly more than the others and so failure at this point has a major impact on the company. The larger numbers of patients included in these studies provide more power for pharmacogenomic analysis. In addition, these samples also provide a useful resource for more disease-focused phenotype–genotype correlations, and often samples are collected with broad consent for genotyping that allows the investigation of many candidate genes.

The patient population studies in the phase III program form the basis of the population approved to use the drug once it is launched. Therefore if a genetically defined patient population is used in these studies, then the drug will only be approved for use in that group of patients. However, it is often necessary to include at least one study where all patient groups are included to ensure that there is not an unexpected benefit in the nonselected population and also to provide a safety database for that group should they be prescribed the drug once it is approved. The inclusion of a prospectively stratified "all comers" strategy also allows a more robust evaluation of the positive and negative predictive value of the test and, importantly, differentiation between a predictive pharmacogenomics test where the test identifies subjects who differentially respond to the drug from a prognostic test where the test-differentiated subjects have a more severe prognosis from the disease regardless of treatment paradigm.

Phase IV

Drug testing does not stop with regulatory approval, and phase IV studies are run after the drug has been approved. Sometimes there are clinical studies required by regulatory authorities as a postapproval commitment. These generally test a specific question around safety and efficacy or are used to generate data to support commercial strategies. Studies conducted after the regulatory approval of the drug represent an excellent resource for the implementation of a pharmacogenomics strategy because of the availability of larger sample sets. The potential to collect genomic samples from thousands of individuals recruited into large phase IV clinical studies presents the opportunity to link genomic data to quality clinical data, biomarker data, and, in many cases, long-term follow-up monitoring. An area where postmarket pharmacogenomic surveillance can have a great impact is in addressing safety issues. The availability of large numbers of patients on active treatments not only provides the material to look for pharmacogenomic effects but is also a valuable resource for understanding the molecular basis for disease, which in turn feeds back into the idea generation in the early discovery section of the pipeline.

The studies performed within drug development programs are still classified according to this system, but, increasingly, companies are looking to generate potential signals of efficacy data in the early phase I and IIa studies (sometimes called the *learn phase*) to provide confidence that the compound will work before investing in the more expensive phase IIb and III studies (sometimes called the *confirm phase*).

APPLYING GENOMICS TO DRUG DISCOVERY

Choosing the Best Drug Targets

One key area where genetics has impacted the drug discovery and development process is in target selection. A significant number of compounds fail in development because the target and hence mechanism of action of the drug is not linked to the pathogenesis of the disease to which they are directed. Taking the view that the more you know about a drug target early in the discovery process, the less likely it is to fail in development caused by lack of confidence in rationale (CIR), many companies are now investing up front in understanding the molecular genetics of the complex diseases we treat and using genetics to identify novel targets and prioritize target selection from candidate gene lists for drug development programs. The advances in DNA sequencing, bioinformatics, and genetic analysis are offering great opportunity to use human genetics to identify novel targets.

Before 1990, pharmaceutical companies had worked on approximately 500 potential drug targets with around 100 of these mechanisms having produced marketed drugs [2]. Initial analysis of the final draft of the Human Genome Project suggested that the total number of drug targets with small chemicals might increase to 5000 [3]. However, not all of these targets will be relevant to disease and therefore current estimates are that there are 600–1500 drug targets in the human genome [2]. This expansion of potential targets in concert with the rising cost of drug development means that the choice of targets is increasingly important.

Given the length of time it takes to get from an idea to a compound to the market, there are few prospective examples of marketed compounds where genomics has provided a new drug target or supported its initial CIR, and thus there is insufficient data to show that having genetic or genomic CIR from complex traits has significantly increased candidate survival in the drug development pipeline. Human genetics is a simple and effective way of beginning to assess the molecular evidence and provide the CIR for establishing a drug development program for a particular target. It is possible to retrospectively identify positive genetic

associations between drug targets and incidence or severity of disease for drugs that are widely prescribed; for example, angiotensin-converting enzyme inhibitors and hypertension [4,5], β-agonists and asthma [6,7], and serotonin reuptake inhibitors and depression [8,9]. However, this is not always the case, because the proton-pump inhibitors, used to treat gastroesophageal reflux disease (GERD) are one of the most commonly prescribed classes of drugs worldwide, but very little is known about the molecular genetics of GERD and there is no reported association between the genes encoding the α and β subunits of the drug target hydrogen/potassium adenosine triphosphatase (ATPase) and the disease [10]. Knockout mouse data also provides evidence relevant to the function of target on the phenotype [11]. The CIR for the statins, one of the most successful drug classes to be developed for the lowering of low-density lipoprotein cholesterol, was derived from biochemistry; the 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase knockout mouse is lethal, and there are very few published genetic association studies on HMG-CoA reductase [12].

Complex Trait Genetics

The ability to carry out large-scale whole genome studies in well-characterized populations extends the candidate gene approach, and has increased the potential to identify novel targets and new pathways that are relevant to disease. Linkage studies have had some success in identifying genetic variants associated with complex diseases; examples include phosphodiesterase 4D (*PDE4D*) and stroke [13], organic cation transporter (OCTN) and discs large homologue 5 (*Drosophila*) (*Dlg5*) genes with inflammatory bowel disease, [14,15] and 5-lipoxygenase-activating protein (*FLAP*) and myocardial infarction and stroke [16]. These studies have provided some supporting evidence for the link between potential drug targets and disease, but rarely as the only evidence supporting this link. This is owing to the fact that the reproducibility of early genetic association studies was poor with many false-positives reported, the identification of the causative variant is often challenging, and hence the prediction of whether the genetic variant is causing an increase or decrease in protein function can be a challenge. Three major advances have occurred in the last decade that has positively impacted the use of complex trait genetics. The first was the publication of the Wellcome Trust Case Control Consortium, which clearly demonstrated the need for larger sample sizes and rigorous quality control procedures [17]. The second has been the rapid development of DNA sequencing, which in 2013 is reaching a point where it is possible to sequence large cohorts of subjects, allowing the evaluation of rare variants as well as the common variants covered by the whole genome association studies [18]. The final advance is that the development of bioinformatics and genetic analysis is allowing the combining of the genetic variations into pathway maps looking for dysregulated pathways rather than just individual single nucleotide proteins (SNPs) [19]. This allows for the identification of optimal intervention points in pathways and the design of functional experiments, confirming the direction of the dysregulation and hence whether an agonist or antagonist approach is required.

Single Gene Disorders/Traits

One key approach to increase the predictivity of genetic data is to use rare genetic syndromes to identify drug targets with high confidence that pharmacological approaches will mimic the human phenotype seen in the family. The last 5 years have seen the first cohort of drugs reach approval or late-stage clinical development where human genetics either identified the target or provided significant confidence in the approach. Examples of these drugs are included in Table 19.1 but include maraviroc and *CCR5* (HIV), tofacitinib and the *JAK* kinases (rheumatoid arthritis), romosozumab and sclerostin (*SOST*) (postmenopausal osteoporosis), and vemurafenib and *BRAF* (melanoma).

The identification of *CCR5* as a potential therapeutic target for HIV infection came from the identification of *CCR5* and the coreceptor for HIV and a genetic study of individuals, who, despite multiple high-risk exposures, did not become infected with the virus. The association between a common mutation in the gene encoding CCR5 that resulted in a nonfunctional protein and resistance to HIV infection identified the *CCR5* receptor as a coreceptor used by HIV to infect cells in the majority of primary infections. Individuals who were homozygous for this mutation (*CCR5Δ32*) and therefore had no functional CCR5 protein

TABLE 19.1 Drugs with Targets Defined by Human Genetics

Drug	Gene	Phenotype	Indication
Maraviroc	*CCR5*	HIV resistance	HIV
Tofacitinib	*JAK 3*	Severe combined immunodeficiency	Rheumatoid arthritis
Romosozumab	Sclerostin	Sclerosteosis	Osteoporosis
Clopidogrel	*P2yR*	Congenital bleeding	Ischemic heart disease
Alirocumab	*PCSK9*	Hypercholesterolemia	Ischemic heart disease
In development	*Nav 1.7*	Insensitivity to pain	Pain

were apparently healthy and resistant to infection by HIV [20]. Subsequent candidate gene studies have shown that the *CCR5Δ32* mutation is associated with slower progression to AIDS [21]. Recent data have shown that a genetic polymorphism in the promoter of the *CCR5* gene, resulting in increased CCR5 expression, is more common in individuals rapidly progressing to AIDS [22]. Thus within 7 years of the publication of genetic evidence that *CCR5* would be a valid target in HIV therapy, clinical validation of this drug target was achieved, with both Pfizer and Schering-Plough publishing data showing significant viral load drops in patients with HIV infection treated with the potent CCR5 antagonists maraviroc and Schering C (vicriviroc), respectively [23].

The discovery of Janus kinase (JAK) and the identification of causative mutations in the *Jak3* gene and severe combined immunodeficiency (SCID) highlighted the key role of this target in cytokine signaling and lymphocyte development and function and provided CIR for the development of a selective *Jak3* antagonist for the treatment of rejection in renal transplantation and rheumatoid arthritis. As with CCR5 above, the fact that individuals with the mutations only have the very specific effects of immunodeficiency and no other apparent deleterious phenotype means that these genetic data also provide confidence in safety (CIS) for the therapeutic approach [24].

Sclerosteosis is a rare genetic condition seen in only a small number of families in the world. A key aspect of the disease phenotype of sclerosteosis is bone overgrowth. This bone overgrowth is seen in the heterozygotes when they have generalized increase in bone density and mass, and the homozygotes when they have increased bone growth and density that can lead to nerve entrapment syndromes causing deafness and visual problems. The gene for sclerosteosis was identified in 2005 and the disease is cause by the absence of a protein called *sclerostin* [25]. Sclerostin is a secreted protein which is highly amenable to a biologics approach and where reduction in circulating sclerostin will lead to increase in one density. This led to collaboration between UCB Celltech and Amgen to produce an antibody to sclerostin for the treatment of postmenopausal osteoporosis. This antibody has now been tested in phase IIb and shown to increase bone mineral density to a greater extent than current therapies.

Drug-Specific Targets Approach

An alternative strategy to the single gene and whole genome approaches is to carry out association studies in a large subset of specific drug target genes. Several companies have taken this approach to explore genetic association with as many targets as possible in many indications. Oxagen is a biopharmaceutical company specializing in understanding the genetic basis of common human diseases. One of the main areas of interest for the company is in G-protein coupled receptors (*GPCRs*); 20–30% of marketed drugs are targeted to the products of this class of genes. There are over 750 *GPCR* genes, thus Oxagen applied a filtering process to select the best targets for further analysis based on expression profiling, known biology, whether they have a known drug targeted to them, or are likely to be chemically tractable before high-throughput genetic analysis [26]. The Structural Genomics Consortium has focused on kinases (the kinome). This consortium is funded by private and public sources and focuses on the identification of crystal structures of novel kinases and then the development of chemical tools. In concert with this, there have been considerable efforts to identify kinases and their role in disease. Much of this has focused on the use of genetic mutations of kinases in cancer and genetic associations in conditions such as rheumatoid arthritis.

With the increasing use of genetics to drive target identification in well-defined patient populations comes the dilemma of knowing which of all the targets identified is the best to take forward. Many of the positive genetic associations with disease from linkage disequilibrium based whole genome association studies (LD-WGA) are likely to occur in noncoding regions of the genome and the basis for a strong association, if replicated, will be unknown. Recent data investigating noncoding parts of the genome have revealed the importance of these regions in regulating gene expression [27].

The application of whole genome technologies to understanding common complex disease has increased the number of potential targets.

Effect of Genetic Variation on Compound Screening

Regardless of the original source of the target, genetic analyses are important in understanding how to move forwards in the drug discovery process. Undertaking a comprehensive analysis of the genetic variation that exists in putative drug targets will provide information that has the potential to impact drug discovery processes downstream. In an internal study within Pfizer comparing coding SNP (cSNP) frequency, a selection of 111 genes encoding potential druggable targets and 160 genes considered to be "nondruggable" targets identified that 15% (26/111) of the putative targets were not polymorphic at the amino acid level, whereas 40% (45/111) had one or two cSNPs. There are also well-documented differences in the frequencies of specific polymorphisms between ethnic groups. Prior knowledge of any polymorphisms in a target can be incorporated into target validation, lead optimization, and inform preclinical projects supporting the development of the compound. The effect of genetic variation can be assessed through in vitro assays that incorporate a comparison of polymorphic targets either by using cells or biological reagents obtained from donors of known genotypes where available,

or by site-directed mutagenesis. This will facilitate early assessment of the potential impact of genetic variation on the activity of compounds and offer the potential to choose candidates that are least likely to be influenced by the target polymorphism [28].

Gaining an early understanding of the impact of genetic variation can increase confidence in chemistry (CIC). For example, chemokine receptor 5 (*CCR5*) has been shown to be the second coreceptor required for primary HIV infection. As such, it was a very attractive drug target for the treatment of HIV, because blockade of *CCR5* should reduce HIV entry into cells and hence lower viral turnover. There have been multiple polymorphisms reported in the *CCR5* gene, and some of these have been associated with effects on HIV infection rates and/or progression from infection to AIDs. A key question that had to be asked was what the functional effects of these polymorphisms were and whether they would impact the effectiveness of the therapy. Preclinical experiments demonstrated that the predominant effect of the functional polymorphisms was to alter receptor expression rather than structure, and hence that the variability could be managed by identifying a dosage that could effectively inhibit viral entry across a wide range of receptor expression levels.

The pharmacogenomic studies included in the preclinical phase of drug discovery that provide CIR and CIC and support nomination of a candidate drug for development are not intended to replace any of the clinical studies required for exploratory drug development or predict response in patient populations. The preclinical strategy will produce data to inform the pharmacogenomic plan for compounds in exploratory and full development. The challenge facing pharmacogenomic specialists in the pharmaceutical industry is to use the available genomic data to improve the efficiency of clinical trials.

APPLYING PHARMACOGENETICS TO DRUG DEVELOPMENT

Despite relatively early introduction, pharmacogenetics (see chapter: Content and Variation of the Human Genome) remained a relatively small field for the next 40 years because of the fact that although it was well recognized that all drugs exhibited significant interindividual variability in response, the genetic tools to examine this variability were not available. Apart from a few standard approaches, for example, renal impairment studies and gender differences, there was limited investigation of this phenomenon during drug development. The approach of the drug companies and regulators alike was to ensure that all compounds had a sufficiently good therapeutic index that the average benefit significantly outweighed the potential risk. This has led to the withdrawal or termination of development of a number of compounds with good efficacy but an insufficient population-based safety profile, which can often be driven by a small number of potentially serious adverse events. These events can be categorized into those that are expected based on an understanding of the pharmacological action of the drug (type A) and those that correlate with plasma exposure levels or idiosyncratic (type B) [29]. The mechanism of idiosyncratic reactions are generally unknown and do not have a clear dose–response relationship. There are two basic pharmacological implications of underlying genetic factors: pharmacodynamic (PD) variability (drug absorption and delivery to desired target), and PK variability (drug metabolism and excretion).

Pharmacodynamic Variability

The importance of being able to predict drug response is highlighted by the fact that it has been estimated that approximately 30% of prescriptions written do not benefit the patient and even in highly controlled environments, such as clinical trials, it is rare to get response rates significantly above 70% [30]. If we assume that subjects take the medication in the prescribed manner, then lack of efficacy may result from inadequate exposure to the drug (PK variability), an inability to respond to the therapy because of genetic variation in the target and/or downstream effectors (PD variability), or because the pharmacological intervention does not alter the underlying pathophysiological process (disease heterogeneity). Whereas some commentators have suggested that differences in disease genetics (disease heterogeneity) should be considered as separate from pharmacogenetics, at a practical level, understanding this genetic variation will result in the same outcome; for example, understanding increased or decreased likelihood of response to therapy. Therefore this group will be included in the PD variability subgroup. There are now multiple examples of the use of pharmacogenetics to predict drug response. The majority of these are in oncology where tumor mutations have been shown to drive PD response in multiple areas. The best known examples of this are trastuzumab and imatinib (Gleevec). In the case of trastuzumab, amplification of the *Her2* gene leads to upregulated Her2 expression in approximately 25% of all breast cancers. These tumors are responsive to trastuzumab, whereas tumors with very low levels of expression of Her2 do not respond. Imatinib is a treatment for Philadelphia chromosome–positive chronic myeloid leukemia (CML) specifically designed to target the BCR ABl fusion protein generated from this chromosomal translocation. It also is active in tumors with mutated *KIT* genes; eg, *GIST*. Table 19.2 contains a list of antitumor therapies aimed at genotypically defined tumors.

Vemurafenib is a very exciting example, because this compound was screened using the common V600E mutation of the *BRAF* gene. The mutation is present in 35–45% of melanoma cases. A counter screen of nonmutated *BRAF*

TABLE 19.2 Selected Drugs: Genotype–Phenotype Correlations

Drug	Indication	Gene
Imatinib	Gastrointestinal stromal tumor	*KIT*
Gefitinib	Non–small cell lung cancer	*EGFR*
Erlotinib	Non–small cell lung cancer	*EGFR*
Cetuximab	Colorectal cancer	*KRAS*
Panitumumab	Colorectal cancer	*KRAS*
Crizotinib	Non–small cell lung cancer	*Alk4*
Vemurafenib	Melanoma	*BRAF*

was also run, ensuring the identified compound was specific for the mutated allele. This drug is highly effective in V600E-positive tumors and has a very good safety profile because it does not bind to the nonmutated protein, hence only working within the tumor cells.

Although the majority of examples are in oncology, there are exemplars in other therapeutic areas as well. One of the clearest examples is in the treatment of hepatitis C subjects who have the AA polymorphism in their interferon gene have a greater chance of responding to interferon therapy. Other examples exist particularly in the rare disease in which therapies are directed at specific genetic disorders and, in this case, it is disease genetics rather than PKs. Despite the success observed over the last 5 years, most therapies tested do not appear to have a clear pharmacogenetic signature. It may be that the current approaches are unable to identify the correct genetic variation or, more likely, the combination of variants that can predict response or that genetic variation is not a major cause of the heterogeneity of drug response.

Pharmacokinetic Variability

Interindividual variation in drug metabolism is now a well-documented phenomenon, but it was not until Mahgoub et al., in 1977 [31] described the polymorphic metabolism of debrisoquine that significant interest grew in the genetic contribution. The cytochrome P450 (CYP) enzyme family protects the body from xenobiotic agents and is the major route of metabolism of many drugs [32]. Several of these enzymes (for example, 2D6, 2C9, and 2C19) are known to have functional genetic polymorphisms that result in significant reductions or increases in function [33,34]. Genetic variation in cytochrome P450 2D6 (CYP2D6) is well characterized and approximately 10% of Caucasians make no 2D6 enzyme. Experiments with the antihypertensive agent debrisoquine were the first proven examples of a pharmacogenetic effect. Debrisoquine is metabolized by the CYP2D6 enzyme. An individual who makes no 2D6 and takes a standard dose of debrisoquine will suffer a profound hypotensive event resulting from high plasma exposure levels caused by an inability to metabolize the drug [35]. Approximately 20% of all drugs are metabolized by 2D6 and subjects who are unable to make this enzyme are at increased risk of developing adverse events when taking one of these compounds [36].

Interindividual variation in drug metabolism is well documented. Approximately 20% of drugs are metabolized by the CYP2D6 enzyme [36]. The incorporation of genetic testing for CYP2D6 or related enzymes in clinical trials has the potential to identify, prospectively, subjects who are likely to have adverse events because of poor metabolism or those who may have limited response through inadequate exposure because of ultrarapid metabolism.

Many drug-metabolizing enzymes have genetic variants, leading to reduced or increased function with consequent impact on the PK variability. Despite this knowledge, there are no drugs for which pharmacogenetic tests are routinely applied, and only recently has it become accepted best practice to test for the presence of variation in the gene encoding the thiopurine methyltransferase (TPMT) enzyme before prescription of azathioprine and 6-mercaptopurine. Approximately one in 300 individuals are homozygous for mutations in the gene encoding TPMT [37]. If treated with a standard dose of azathioprine (6-mercaptopurine), these individuals have a substantially increased risk of developing the potentially fatal complication of red cell aplasia [37]. Suitable dosage reduction decreases this risk. The recent decision by the clinical pharmacology division of the US Food and Drug Administration (FDA) to recommend that subjects be tested for TPMT enzyme status (either phenotypically or genotypically) before receiving a dose of 6-mercaptopurine is evidence of the increasing awareness of the value of understanding interindividual variation in drug metabolism. Similarly, the recently approved drug atomoxetine (Strattera) from Eli Lilly provides safety data for CYP2D6-poor and CYP2D6-extensive metabolizers, and the availability of a suitable test to distinguish these two groups is also included on the label, although there is no recommendation about using the test and adjusting the dosage according to genotype.

As the clinical value of these tests becomes established and is translated into practice, so will the acceptability of requiring a metabolizing enzyme diagnostic test before dispensation of the drug. Clear demonstration of the advantages of prospectively using a diagnostic test versus clinical management of drug dosaging will also be vital if these tests are to be used in clinical practice. This will also allow the development of chemicals with narrow therapeutic

windows and predominantly metabolized by a polymorphic enzyme. Many of these compounds have historically been terminated, as the risk of adverse events caused by high plasma exposures outweighed the potential benefit. A clinically acceptable way of managing this risk would make safe use of these compounds possible.

PREDICTING SAFETY

Predicting Type B Adverse Events

The last 5 years has demonstrated that pharmacogenetics can be used to predict some rare adverse events. Extreme PD adverse responses to drugs have been described in the past; eg, malignant hyperthermia and inhaled anesthetics, and succinyl choline deficiency and prolonged paralysis. More recently an immunogenetic explanation for rare hypersensitivity reactions was discovered. Abacavir (Ziagen) was a key drug in highlighting the role of *HLA* variation and drug hypersensitivity. Two retrospective studies have identified the *HLA*-B*57:01 allele of the major histocompatibility complex (*MHC*) class I B gene as a genetic determinant of hypersensitivity to abacavir [38,39]. The availability of a relatively large patient population led to the identification of the *HLA*-B*57:01-Hsp70-Hom variant haplotype in 94.4% of cases compared with only 0.4% of controls. Analysis in different ethnic groups, however, showed that *HLA*-B*57:01 alone would not be sufficiently predictive of hypersensitivity in diverse patient populations, suggesting that other genetic determinants of hypersensitivity remain to be identified. Additional *HLA* associations with adverse drug reactions (ADRs) have been described. Chung et al., in 2004 [40] described an association between *HLA*-B*1502 and Stevens-Johnson syndrome in Han Chinese. Again, this association appears to be confined to the Han Chinese. Additional *HLA* associations and immune mediated adverse events have since been confirmed (Table 19.3).

Predicting Type A Adverse Events

ADRs are a major cause of morbidity, leading to approximately 5% of all hospital admissions, and severe ADRs are a leading cause of death in young adults. Despite initial optimism, pharmacogenetics has had limited impact in reducing this morbidity and mortality. Genetic variation can influence our risk of developing type A adverse events by either increasing our exposure to the active agent or altering the PD effects of the drug. Warfarin is one of the best understood examples of how genetic variation can influence risk of adverse events. Bleeding events

TABLE 19.3 Drug Response Modification Associated with Genetic Polymorphisms in "Disease-Modifying" or "Treatment-Modifying" Genes

Gene or Gene Product	Disease or Drug Effect	Medication	Influence of Polymorphism
Adducin	Hypertension	Diuretics	Myocardial infarction or stroke
Apolipoprotein E (*APOE*)	Atherosclerosis, ischemic cardiovascular events	Statins (simvastatin)	Enhanced survival
Apolipoprotein E (*APOE*)	Alzheimer disease	Tacrine	Clinical improvement
HLA	Toxicity	Abacavir	Hypersensitivity reaction
Cholesterol ester transfer protein (*CETP*)	Progression of atherosclerosis	Statins (pravastatin)	Slowing of atherosclerosis
Ion channels (*HERG, KvLQT1, Mink MiRP1*)	Congenital long QT syndrome	Erythromycin, cisapride, terfenadine, clarithromycin, quinidine	Increased risk of drug-induced torsade de pointes
Methylguanine methyltransferase (*MGMT*)	Glioma	Carmustine	Response of glioma
Parkin	Parkinson disease	Levodopa	Clinical improvement and levodopa-induced dyskinesias
Prothrombin and factor V	Deep-vein thrombosis and cerebral vein thrombosis	Oral contraceptives	Increased risk of deep-vein and cerebral-vein thrombosis
Stromelysin-I	Atherosclerosis progression	Statins (pravastatin)	Reduction in cardiovascular events: death, myocardial infarction, stroke, angina; reduction in risk of angioplasty

Adapted from Evans WE, McLeod HL. Pharmacogenomics: drug disposition, drug targets, and side effects. N Engl J Med February 6, 2003;348(6):538–549.

while taking warfarin is one of the most common adverse events resulting in significant morbidity. Underlying genetic variation accounts for at least 50% of the risk of developing a bleeding event. This risk is predominantly driven by two key genes: the drug-metabolizing enzyme *CYP2C19* and the gene encoding the vitamin K receptor. Studies by Lane et al. [41] have shown that poor metabolizers' status of cytochrome *CYP2C19* have a approximately four fold increase in plasma exposure of S-warfarin (the active moiety). The increase in exposure results in a five fold increase in bleeding risk caused by PK variability. The vitamin K receptor is the target for warfarin and required for the production of vitamin K–dependent clotting factors. A common variant in this receptor results in a decrease in vitamin K receptor function. Whereas this normally causes no significant sequelae, it does impact response to warfarin. Individuals who are homozygous for the rare allele have an increase in bleeding risk of approximately two fold when taking warfarin. By combining the results of these genotypes, it is possible to refine an individual's risk of developing a bleeding adverse event if they are given a standard dose of warfarin. Prospective trials are now ongoing to determine the utility of using genotype results to adjust the starting dosage of warfarin.

Individualized Therapy: An Integrated Response

In real life, the response of an individual is based on both the plasma exposure and how that affects the various physiological processes in the target organs. Evans and Relling generated a hypothetical graph representing the PK and PD variation in concert [42].

Variation in drug metabolizing enzymes can dramatically impact plasma exposure levels. However, it is not until we integrate this with variation in genes affecting PD response in the right hand column that we start to get a real understanding of the impact on response for the individual. It is important to realize that dosage-related adverse events are observed in extensive metabolizers as well as poor metabolizers, but the incidence is dependent upon the frequency of variation in the genes affecting PD response. As the frequency of variation in genes affecting PD response approaches 0.5, the predictive power of a test solely looking at drug metabolism decreases. Similarly, the predictive power of a test evaluating variation in genes impacting PD response will vary depending upon PK variability. Most published pharmacogenetic studies concentrate on single genes or small numbers of candidate genes, which are likely to impact either PK or PD variability. It is unsurprising that these studies fail to demonstrate high positive or negative predictive information for drug response that is in general caused by a combination of both of these factors. As we move forward, a more holistic approach to the examination of genetic factors impacting drug response should lead to the identification of sets of SNPs with higher predictive values, leading to improved prescribing (Table 19.1).

Improving Disease Classification: Stratified Medicines

The need to accurately and precisely characterize the disease under investigation has important implications in drug development. The current disease classification system has changed little in the last 100 years and is based on the phenotypic clustering. That is, diseases that present with similar symptoms have been classified as having the same condition. These diseases are therefore more like syndromes and do not necessarily reflect a common underlying pathology. Likewise, there may be conditions with similar pathological mechanisms that are classified as different diseases because the phenotypic features are not similar enough. A very clear example of this is in oncology, where many mechanisms are represented in subsets of organs classified tumors; eg, *EGFR* mutations are present in multiple tumor types. The knowledge from the outset of a drug discovery program that there are molecular subtypes of disease means that appropriate preclinical experiments can be developed early to predict the likelihood of a pharmacogenomic effect, and this information can be used advantageously in the drug development program. Combining genotype data with other genomic data provides valuable information related to disease subtype. Integration of genotyping data with gene expression has identified subtypes of obesity phenotypes in a mouse model [44]. Using similar approaches and including microRNA, epigenetic, proteomic, and metabonomic analyses in well-defined patient cohorts will provide powerful tools to aid the dissection of the phenotype of disease in humans in order to drive the development of targeted therapies based on molecular subclassification. This reclassification of disease has become the focus of several cross-academic/industry consortiums, and the next decade could see the development of new disease taxonomies reflecting the true molecular mechanisms of the pathology rather than the end consequences.

One therapeutic area where using genetic and genomic technologies has undoubtedly had a major and measurable impact on understanding the molecular subtypes of disease is oncology. The advances in understanding the molecular mechanisms predisposing a patient to cancer have seen the number of oncology compounds in clinical development rise from 10 to over 400 in 10 years. The majority of the new compounds now being tested are classed as targeted biotech medicines. Imatinib and trastuzumab were the first two such targeted compounds approved. Trastuzumab is a therapy targeting the *HER2/neu* receptor in breast cancer. The rationale for this therapy was based on a sound understanding of the underlying molecular pathology. It was known that only 20–30% of breast tumors overexpress this

protein and it was demonstrated in the drug development program that response to trastuzumab was limited to subjects whose tumors overexpressed the target [45]. Similarly, imatinib is a therapy targeting the fusion protein product resulting from the Philadelphia chromosomal translocation observed in most cases of CML [46]. This therapy provided dramatic efficacy in cases of CML with the chromosomal translocation and was rapidly approved by the FDA.

Since the rapid approval and success of imatinib and trastuzumab, many other targeted cancer therapies have entered clinical trials, thus highlighting the absolute requirement to continue to investigate and understand the underlying molecular mechanisms that are associated with disease. Gefitinib (Iressa) was the first of class-selective EGFR inhibitors to receive accelerated approval based on preliminary data from phase II studies in non–small cell lung carcinoma (NSCLC) patients. Activating mutations and overexpression of EGFR were known to occur in many cancers, providing CIR for development of an EGFR inhibitor for cancer treatment. Inactivation of the *EFGR* gene in mice did not cause any major phenotypic effects, which, in turn provided CIS with respect to pharmacological inhibition of this target [47]. However, tumor response to treatment in the clinical trials was only observed in 9–19% of patients. Subsequent analysis to predict factors that would indicate good response to gefitinib identified that female gender, nonsmoking status, and specific histological subtype of tumor was associated with better response to therapy. Investigation of biological and markers of response failed to show an association with EGFR expression levels. However, somatic mutations in the ATP-binding site of the tyrosine kinase domain of EGFR were observed more often in the tumors of patients who responded to gefitinib. The *EGFR* mutations are located close to the putative binding site for compounds like gefitinib and lead to increased signaling in the growth factor pathway, and thus tumors harboring these mutations are more susceptible to treatment with an EGFR inhibitor [48]. This highlights the importance of defining the molecular subtypes of disease and understanding the impact on response to therapy. Had the molecular profile of NSCLC been identified before testing in humans, it may have been possible to design preclinical cell-based assays to determine whether the genetic profile of the tumor would influence response to therapy and then inform clinical trial design.

The majority of oncology programs now in development are focusing on stratified populations based on genetic or genomic classifications of tumor type.

Adverse Drug Reactions

In a recent study of ADRs, 5% of hospital admissions in the United Kingdom were identified as being the result of ADRs. Over 70% were considered avoidable, and whereas drug interactions accounted for the majority of the ADRs and older drugs were implicated in the hospital admission, there is still a need to understand the underlying causes of all ADRs [49]. It is difficult to detect rare adverse events in the confines of a clinical trial and the current system for monitoring ADRs has been suggested to be too disparate. A move to a more comprehensive epidemiological approach to monitoring drug safety has been proposed. The inclusion of pharmacogenomic analyses within this approach would allow the systematic assessment of the contribution of genetic determinants to ADRs. Pharmacogenomic surveillance in large phase IV trials of approved compounds will have a great impact in addressing safety issues.

One therapeutic area where detailed pharmacosurveillance, including pharmacogenomic analyses and postapproval, is not new is in the antiretroviral treatment of HIV infection. Viral resistance and drug toxicity are common and often lead to treatment failure. Determination of HIV genetic sequences and viral load are constantly monitored to assess viral resistance to highly active antiretroviral therapy. Polymorphisms in drug transporters and drug metabolizing enzymes have also been monitored in HIV therapy. Two retrospective studies have identified the *HLA*-B*57:01 allele of the *MHC* class I B gene as a genetic determinant of hypersensitivity to abacavir [38,39]. Analysis in different ethnic groups, however, showed that *HLA*-B*57:01 alone would not be sufficiently predictive of hypersensitivity in diverse patient populations, suggesting that other genetic determinants of hypersensitivity remain to be identified. Implementation of pharmacogenetic postapproval will have a role in increasing the CIS of new products.

SUMMARY

The genomic revolution has offered the pharmaceutical industry the potential of improving the efficiency of drug development by reducing the current high failure rate through better choice of targets and improved understanding of drug response early in development. To the healthcare providers, it offers the potential to reduce the burden of adverse events by identifying those subjects at increased risk and offering them alternative therapies, as well as targeting its resources to use newer, more expensive treatments on subjects who will derive most benefit. Finally, and most importantly, it offers to the patient the opportunity with their physician to identify from the range of available therapeutic options the one most suited to them. Although pharmacogenetic testing is unlikely to be able to guarantee that the therapy will work and will not cause an adverse event, it will increase the probability that a drug will work and reduce uncertainty around adverse events and provide a rational way of choosing between therapies.

As our understanding of genomics improves, so will our ability to determine key factors involved in variability of drug response. The quest for precision medicines will start at the beginning of the drug discovery process with more comprehensive understanding of the molecular basis of the disease, molecular stratification, and the role of the drug target in the pathological process. Significant PK variability will be explained by systematic evaluation of all the relevant metabolizing enzymes and transport proteins. The drug candidates will only be tested in patients with suitable variants of the drug target. Drugs will be approved with variable dosage levels dependent upon underlying genotypes affecting drug response variation at the desired drug target. Finally, genetic- and genomic-led drug development and evaluation will not stop with the approval, but postmarketing (phase IV) research will endeavor to identify the causes of uncommon adverse events, leading to continuous refinement of how we use drugs throughout their life cycle.

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