Guidance for Industry

Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling

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U.S. Department of Health and Human Services
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Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
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I.   INTRODUCTION

This guidance is intended to assist the pharmaceutical industry and other investigators engaged in new drug development in evaluating how variations in the human genome, specifically DNA sequence variants, could affect a drug's pharmacokinetics (PK), pharmacodynamics (PD), efficacy, or safety.[2] The guidance provides recommendations on when and how genomic information should be considered to address questions arising during drug development and regulatory review.

The application of pharmacogenomic approaches during drug development is an evolving process that begins with discovery and continues through confirmation of clinical efficacy and safety outcomes. The focus of this guidance, however, is to provide advice on general principles of study design, data collection, and data analysis in early-phase trials. This guidance does not address trial design or statistical analysis considerations for later-phase, randomized, controlled clinical trials that are intended to draw definitive conclusions about treatment effects in a genomic subgroup (e.g., enrichment designs, adaptive enrichment designs, simultaneous hypothesis testing overall and within subgroups), or co-development of a drug and in vitro diagnostic.

Rather, the considerations here are more relevant for exploratory and observational studies intended to generate genomic hypotheses that may then be tested in prospectively designed phase 3 trials. For instance, early-phase data on genomic-dependent dosing or efficacy, even when not definitive, can provide guidance on dosing or patient selection in later-phase trials, or inform the strategy for further collection of genetic and related biomarker data in later controlled trials.

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[1] This guidance has been prepared by the Office of Clinical Pharmacology, Office of Translational Sciences, and an Interdisciplinary Working Group with representatives from the Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), and the Center for Devices and Radiological Health (CDRH) at the Food and Drug Administration (FDA).

[2] For the purposes of this guidance, the term *drug* includes both small molecule and biological products.

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II. BACKGROUND

Pharmacogenomics (PGx) refers broadly to the study of drug exposure and/or response as related to variations in DNA and RNA characteristics (see the International Conference on Harmonization (ICH) E15 *Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories*).[3] *Drug exposure* refers to the PK profile following administration. *Drug response* refers to the PD response to the drug; that is, all of the effects of the drug on any physiologic and pathologic processes, including those related to effectiveness and those related to adverse reactions.

PD effects are generally characterized by an exposure-response (E/R) relationship of drugs, and genetic differences can lead to changes in the steepness of the E/R curve, the location of the curve (i.e., change in EC_{50}), the maximum effect (E_{max}), and other features of the E/R relationship. The definition of PGx in this document focuses mainly on DNA sequence variations and is not intended to include other related characteristics, such as proteomics and metabolomics.[4]

A. Genetic Differences

Genetic differences between individuals can affect virtually all aspects of a disease and its treatment, including the rate of disease occurrence; the risk of disease progression or recurrence; the drug or drug class most likely to provide benefit; the therapeutic dose; the nature and extent of beneficial responses to treatment; and the likelihood of drug toxicity. The genetic differences likely to be of most relevance in drug development are those associated with genes in four broad categories: (1) genes relevant to the drug's PK (absorption, distribution, metabolism (including formation of active metabolites), and excretion (ADME)); (2) genes that code for intended or unintended drug targets and other pathways related to the drug's pharmacologic effect; (3) genes not directly related to a drug's pharmacology that can predispose to toxicities such as immune reactions; and (4) genes that influence disease susceptibility or progression. All of these genetic factors can affect the benefit–risk profile of a drug product.

In general, the effects of genetic differences on PK are easier to characterize because they affect a readily measured feature of the drug (its pharmacokinetics). There are far more examples of

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[3] FDA guidances, including ICH guidances, are available on FDA's guidance Web page. We update guidances periodically. To make sure you have the most recent version of a guidance document, check FDA's guidance Web site at FDA Basics for Industry > Guidances.
(http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234622.htm).

[4] For the purposes of this guidance, the terms *genetic* and *genomic* are used interchangeably.

genetic PK differences than examples of genetic differences on PD in most therapeutic areas. In many cases, the mechanism that causes differences in PK is related to metabolizing enzymes or transport proteins with well-established genetic polymorphisms, as is the case for *CYP2C9*, *CYP2C19*, *CYP2D6*, or *SLCO1B1*, so that such differences can be anticipated. In these cases, DNA sample collection, blood and/or urine drug concentration data, and well-characterized phenotypic information are needed to determine the extent to which genetic polymorphisms in metabolism and/or transporter genes influence exposure to drugs and/or active metabolites and responses.

In contrast to genetic differences affecting PK, genetic differences affecting PD are more difficult to detect because clinical effects are more complex traits, generally more variable among individuals and complicated by variability over time within the same individual. Clinical effects may also be influenced by many factors, including imprecision in measurement and differences in drug exposure. Genomic effects on PD can, however, profoundly affect selection of the optimal dose based on differences in dose- or exposure-response, safety, and efficacy. For example, in addition to genetic variants in its metabolizing enzyme *(CYP2C9)*, genetic variations in warfarin's target (*VKORC1*) significantly affect response and dose requirements (see warfarin product labeling for details).

Drug product labeling has increasingly included information obtained during drug development on the treatment effect or likelihood of treatment response in a subset of patients with a particular genetic/genomic status, on altered risk–benefit balance in genetic subgroups, or on the need to genotype to guide dosing. Drug product labeling has also been revised after approval, generally based on postmarket experience, to include PGx information that can inform the benefit–risk relationship or allow dosing of the drug to be adjusted for individuals (see Appendix for select examples). It is hoped that ascertainment of genomic information throughout drug development will enable earlier discovery of clinically important genomic differences (i.e., before marketing).

B.   Pharmacogenomics Studies

PGx studies can contribute to a greater understanding of interindividual differences in the efficacy and safety of investigational drugs. PGx research depends on the collection and use of biological samples to generate data.

Across the drug development continuum, genomic data may be used for several purposes, including (1) identifying the basis for PK outliers and intersubject variability in clinical response; (2) ruling out the role of polymorphic pathways as clinically significant contributors to variable PK, PD, efficacy, or safety; (3) estimating the magnitude of potential drug–drug interactions; (4) investigating the molecular or mechanistic basis for lack of efficacy or occurrence of adverse reactions; and (5) designing clinical trials to test for greater effects in specific subgroups (i.e., use in study enrichment strategies) (Zineh and Pacanowski, 2010).

Drug development is commonly described in *phases* (21 CFR 312.21). The first two phases provide initial information about safety and efficacy and ideally examine a broad range of doses, so that the larger, later adequate and well-controlled trials (phase 3) that are generally needed to support marketing approval can be efficiently designed (e.g., with a narrower and reasonable

range of doses, good patient selection criteria, and appropriate endpoints). In addition, information about PK and pertinent PD effects (generally effects on biomarkers considered indicative of activity and/or effectiveness) can provide proof-of-concept, which may improve success of later trials. Information on PD often includes evidence of E/R (usually dose-response but sometimes supplemented by concentration-response modeling) and, when possible, pertinent subset information (e.g., demographic, disease severity) that may improve the design of phase 3 trials by refining dose selection and identifying patients with potentially greater responses.

PGx assessment in early-phase clinical studies may:

1. Identify populations that should receive lower or higher doses of a drug, or longer titration intervals, based on genetic effects on drug exposure, dose-response, early effectiveness, and/or common adverse reactions. Often, these differences are related to differences in drug absorption, distribution, excretion, or metabolism. The latter are generally identified by genetic variations in polymorphic enzymes that define metabolic status. However, genetic factors that affect dose- or exposure-response relationships also could help define the dose range for later trials.

2. Identify responder populations based on phenotypic, receptor, or genetic characteristics, a critical element in treatment individualization that has been used primarily in the oncologic setting. Predicted differences in response can lead to enrichment strategies based on such predictive markers.

3. Identify high-risk groups. Drugs that cause serious adverse effects generally will not be acceptable in most settings, unless the adverse effects can be predicted. There are examples of genetic markers (typically in immunology-related genes like those in the *HLA* family) that effectively predict susceptibility to serious adverse reactions (see Appendix). It is possible to link adverse drug reactions to genetic factors (e.g., metabolic, immunologic) and manage them in later trials, supporting approval of drugs with particular value, such as those that address an unmet medical need. To date, the most likely use of such information would be to identify poor metabolizers or ultra-rapid metabolizers (e.g., *CYP2D6*) whose blood levels of parent or relevant metabolites could be markedly affected — in trials, they could be excluded or their doses modified to account for genetic variations. Genetic information in early-phase studies can also be useful in characterizing the maximum tolerated doses.

The phase 1 and 2 studies considered in this guidance are often described as *exploratory* in that they are not intended to provide the definitive evidence of safety and effectiveness needed to support drug approval. Nonetheless, PGx studies can provide mechanistic support and be applied in the design or analysis of later trials, potentially improving their efficiency or likelihood of success if the genetic factor can help predict the likelihood and magnitude of response.

Genomic analyses are also common in phase 3 trials (although often not prespecified), and responses can be examined in relation to genotype. In most cases, such post hoc analyses of efficacy will be considered exploratory, but in certain cases, strong subset findings of

significantly altered benefit–risk balance have been included in labeling (e.g., *KRAS* mutations with cetuximab and panitumumab indicated for colon cancer). PGx studies in phase 3 trials can also provide valuable information on the efficacy and safety impact of genetic effects identified in early-phase studies.

Phase 2 studies that suggest genomic influences can lead to phase 3 trials that incorporate findings into prespecified hypotheses. Examples might include enriching the study with genomically defined individuals, determining a dose based on demonstrated variability in earlier studies, or defining a priori hypothesis testing of a primary endpoint in a genomic subset. Decisions to pursue prospective strategies in phase 3 trials will depend on a variety of factors, such as the clinical context, availability of alternative treatments, and the risk–benefit profile of the drug. When prospective strategies to apply genetic information to the use of a drug are planned, early consultation with the appropriate centers (i.e., CDER, CBER, and/or CDRH) is highly recommended.

It is FDA's position that if a *companion diagnostic* is required for therapeutic selection, an FDA-approved or -cleared test will be required at the same time that the drug is approved.[5] An in vitro PGx test would be considered a companion diagnostic device if it will provide information that is essential for the safe and effective use of a therapeutic product as directed in labeling.

III.   PROSPECTIVE DNA SAMPLE COLLECTION

This section of the guidance specifically pertains to collection of DNA samples from appropriate tissue sources (e.g., blood, buccal) for analysis of *germline* (i.e., inherited) gene variations as opposed to acquired somatic mutations (e.g., tumor mutations). This section focuses on DNA specimens; nevertheless, many of the principles discussed may apply to other genetic materials obtained from human tissues, such as RNA or proteins, for which pre-treatment expression patterns may be used to characterize a disease and predict patient's therapeutic response or prognosis.

An important prerequisite to successful use of genetic information in drug development is the appropriate collection and storage of DNA samples from a large enough number of participants in clinical trials, both exploratory studies and the adequate and well-controlled trials intended to demonstrate effectiveness and safety. Ideally, baseline DNA samples should be collected from all patients in all arms of clinical trials in all phases of drug development. The voluntary and incomplete nature of many exploratory genetic studies often raises concerns about potential bias and statistical power, which could compromise the scientific rigor of such studies. Because premarket clinical trials represent the largest and richest source of controlled clinical data for drugs, the drug development and public health value of DNA banking and importance of complete data should be recognized and efforts made to collect baseline genetic information when appropriate.

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[5] FDA has issued a draft guidance for industry and FDA staff *In Vitro Companion Diagnostic Devices*. Once finalized, that guidance will reflect the Agency's thinking on this topic.

When known genetic factors are likely to influence the efficacy, safety, or dosing of the investigational drug, the comparator, or the background therapy, DNA should be collected from all subjects to specifically test those genetic factors for subgroup analysis. Specifically, genomic objectives should be stated in the protocol and described in the informed consent form as applicable in light of the known properties (e.g., metabolism, mass balance, E/R relationship) of the investigational drug or the comparator, including genomic objectives related to drug metabolizing enzyme variants; clinically relevant transporter variants; drug target or signaling protein variants that clearly affect activity or expression; or biomarkers for disease prognosis or response to background therapy.

Potential PGx differences in efficacy and/or safety can arise from genetic variants that are not yet as well characterized as the metabolism or transporter genes and, thus, not anticipated in advance. Therefore, general DNA sample collection for exploratory analyses should be a routine consideration, and is strongly encouraged (or plans are). It then becomes possible to seek explanations for differences in PK, PD, efficacy, tolerability, or safety that were not anticipated prior to beginning the study, noting, of course, potential multiplicity and bias issues.

In some situations, DNA collection may ultimately have limited utility and, therefore, may not be needed. This may be the case, for example, for trials evaluating topical drugs for which systemic exposure is expected to be low. However, collecting DNA to characterize the role of genetic factors is particularly important for drugs with high intersubject variability in PK or PD; bimodal or trimodal distributions for measured PK or PD parameters; observed PK or PD differences between racial or ethnic groups; narrow therapeutic ranges; or potential safety issues (e.g., structural safety alerts, QT prolongation, liver enzyme abnormalities). Because many of these signals are not evident until a large number of subjects have been exposed, plans to collect DNA should be considered at the outset of a drug development program. When possible under applicable laws, regulations, and ethics committee polices, consent for DNA collection for exploratory analyses should be obtained from all participants in all arms of clinical trials. FDA recognizes that clinical trials are often conducted globally, and international regulations and ethics committee policies vary with respect to collection and storage of genetic material (Warner et al., 2011; Ricci et al., 2011).

When complete sample acquisition is not possible, efforts should be made to obtain as high a sample acquisition rate as possible, with additional target collection of DNA samples from those subjects who are identified as PK or PD outliers, who experience a clinical endpoint of interest, or who experience a severe or treatment-limiting adverse event. In cases of incomplete sample acquisition, the specific reasons should be described (e.g., in the applicant's study report) and any potential bias estimated when possible.

DNA collection and storage plans should be specified before initiation of a study to minimize the potential for sample selection bias, even if these samples are studied only at a later time during or after a study. An effort should be made to collect genetic samples at enrollment and/or at baseline to avoid potential bias associated with delayed collection (e.g., selection of tolerant or responsive subjects). This is particularly important when many patients do not complete the study, do not comply with the protocol, withdraw from the trials before experiencing a clinical outcome, or die during the trial. For drugs used in acute care settings, it may not be feasible to

collect DNA on a voluntary basis (i.e., using a separate consent), nor would a genomic biomarker be easily applied in practice. DNA collection in this scenario should still be considered for supportive analyses if indicated, but the timing of consent and sample collection may occur later in the trial as appropriate to the clinical scenario.

Because historically many pharmacogenomic relationships have been identified several years after the drug has been marketed, DNA should be retained in the event that new genomic issues arise after the completion of the studies when possible under applicable laws, regulations, and ethics committee polices. Samples should be retained for a time period that will permit postmarket analysis should the need arise (e.g., at least 15 years). Alternatively, when data are generated from higher-throughput technologies (e.g., genome-wide arrays, ADME chips, and high-throughput sequencing), data appropriate to the study objectives may be retained instead of samples, when possible under applicable laws, regulations, and ethics committee polices. As a general matter, sample retention is preferred in the event the need arises to re-assay the samples.

Routine collection of DNA samples in phase 2 or 3 trials could provide applicants with an opportunity to investigate the causes of lack of efficacy or the occurrence of toxicity in different individuals, using such approaches as exploratory genome-wide association investigations and candidate gene or targeted pathway analyses. So, although the need for genomic tests and possible relationship to dosing, efficacy, or safety may not be suspected at study initiation, they may become of interest at a later time. This is especially relevant when such samples can be analyzed along with samples from other similar studies. Therefore, whenever possible, informed consent procedures should anticipate this possibility. Attention should be paid to the appropriate sample collection, handling, storage, and retention to enable possible exploration of genotype-phenotype relationships after study completion.

Samples that can be used for DNA analysis include a range of biological materials such as blood or buccal cells. In addition to germline mutations, there are also somatic or acquired mutations to be considered, for example, in biopsies from tumors. Like germline mutations, not only can somatic changes be related to drug response, but they may also predict the severity of a disease and disease prognosis (e.g., likelihood of metastasis) and can be used to identify subgroups of patients most suited for outcome trials because of a relatively large rate of events (prognostic enrichment). There are, for example, a variety of breast cancer markers that can predict likelihood of recurrence, a potentially critical selection factor for an adjuvant trial. Information to support the quality and integrity of DNA during sample collection and storage, along with information to show that the DNA material can be used for consistent and reproducible analysis, should be provided in an applicant's study report.

IV.   CLINICAL EVALUATION OF PHARMACOGENOMICS

A.   General Considerations

The value of DNA sample collection and the information that analysis of these samples can provide will vary for different drugs and indications. Known PGx factors identified during nonclinical assessments that affect PK or PD should be considered, particularly when the

threshold between activity and toxicity is narrow. Exploratory human PGx investigations generally begin with in vitro studies, followed by clinical pharmacology studies in humans to assess the PK and PD properties of the drug that might be associated with gene variants in metabolizing enzymes, transporters, or drug target receptors. For example, if in vitro studies show that a molecule's metabolism in human cell systems relies on a well-established polymorphic gene, such as *CYP2C19*, and metabolism is a major route of elimination in humans, it would almost always be important to determine the contribution of genomic factors to variability in PK. These data inform decisions about whether subsequent clinical studies need to take PGx differences into account (e.g., in dose-response studies). When exposures are variable and exposure is correlated with response, these findings may subsequently translate to dose selection.

Various technology platforms are available to rapidly characterize the contribution of established and evolving allelic variations of hundreds of metabolism and transporter genes simultaneously in clinical pharmacology studies to generate hypotheses and better understand variability in PK and/or PD related to genomic factors. Candidate gene approaches are reasonable when a drug interacts with receptors and other targets that have known functional polymorphisms, or when the drug is subject to metabolism or transport by a protein that has known functional polymorphisms. When the pharmacology of the drug (i.e., mechanism of action or metabolism/transport) is not well-characterized, high-throughput platforms (such as ADME gene or genome-wide arrays or high-throughput sequencing) should be considered. Because higher-throughput technologies may generate false positives, it is important to confirm the role of a newly identified marker with experimental evidence, such as in vitro enzyme or transport studies.

To design informative studies and interpret study results appropriately, careful attention should be paid in clinical pharmacology studies to differences, if known, in the prevalence of ADME-related gene variants among racial or ethnically distinct groups. The genetic markers selected for analysis should be appropriate to the population being studied. In smaller studies, it is prudent to include rarer functional alleles for evaluation so subjects are not misclassified because a particular (rare) allele was not tested. Drug-metabolizing phenotype assignment based on genotypes should be carefully considered in clinical pharmacology studies.

B.   Clinical Pharmacogenomic Studies

In vitro studies of metabolism, transport, or drug targets could help identify the need for human PGx studies and contribute to the design and analysis of those studies. The following types of clinical pharmacology studies provide opportunities to prospectively integrate PGx factors for assessing interindividual variability and its implications for subsequent clinical studies. Additionally, retrospective analysis of individual or pooled studies (e.g., of similar design, independent subjects) can be performed to characterize genetic effects on PK or PD. In later-stage phase 3 clinical trials intended to support efficacy and safety conclusions in genomic subsets, stratified randomization or planned subset assessment would generally be expected.

1.   *PK and PD Studies in Healthy Volunteers*

Contains Nonbinding Recommendations

Single- and multiple-dose PK studies provide important initial information on drug PK and can suggest the level of interindividual variability in PK that can be expected in later trials. These studies can provide information on common gene variants affecting ADME, and collection of DNA samples from all participants is recommended so that analysis can be performed on individual subjects to retrospectively evaluate the causes of PK outliers and to help explore the PK parameter distribution.

When there are serious concerns about the toxicity of an investigational drug due to excess exposure at higher doses in individuals with genetically mediated alteration in metabolism (e.g., for drugs with anticipated dose-limiting toxicity and polymorphically mediated metabolism), prospectively genotyping subjects will identify those subjects who are at risk, so that they can (1) receive lower doses or (2) be excluded from PK studies. Subjects with variant genotypes can then be enrolled after there is a better understanding of the in vivo relevance of the metabolic pathway and the relationship between drug exposure and safety. For example, in phase 1 oncology studies designed to characterize the maximum tolerated dose, it would be important to understand whether excess exposures are restricted to subjects with certain genotypes so that that these subjects do not inappropriately cause a generally suboptimal dose to be selected as the maximum tolerated dose — a critical consideration for determining which doses to carry forward to subsequent trials.

When in vitro and/or mass balance studies suggest that an investigational drug is metabolized to a large degree by a polymorphic pathway (e.g., *CYP2D6*), PGx analyses should be conducted in single- and/or multiple-dose PK studies in healthy subjects to evaluate common gene variants with known phenotypic effects to determine the extent of variability and the maximal differences in systemic exposure between genotypes. In most cases, genotyping can be performed retrospectively (after completion of the studies) to evaluate observed variability in PK and PD if relevant PD measurements are available. Because many clinical pharmacology studies may be individually underpowered to definitively characterize the effects of gene variants on PK or PD, even those that are common, a pooled analysis of independent subjects from multiple studies with similar protocols may be necessary.

In some situations, it may be advisable to conduct dedicated clinical pharmacology studies with balanced, prospective, genotype-based enrollment, or to target enrollment of subjects with variant genotypes to permit meaningful retrospective analyses. Such strategies may be appropriate when exposures are strongly correlated with response or safety and the genetic factors expected to affect metabolism or transport are not common enough in the general population to be evaluated in an individual clinical pharmacology study. Also, it may be important to quantify the PK or PD differences between genotypes in a controlled manner after clinically relevant pharmacogenetic effects have been observed. When considered in the context of population PK and other analyses, such studies may help define genotype-specific dosing strategies.

Special consideration should be given to a drug for which conversion to an active metabolite from an inactive parent compound occurs through a polymorphic metabolism

pathway. It is advisable to characterize the metabolism of the drug and study the biological activity of the relevant metabolites early in drug development. In these cases, differences in metabolite exposure among individuals may have implications for dosing, efficacy, and safety. When drug metabolites have important clinical effects (e.g., clopidogrel and codeine), failure to form the active metabolite (e.g., in poor metabolizers) may have profound effects on response while overproduction (e.g., in ultrarapid metabolizers) may result in untoward toxicities (e.g., codeine). It is particularly critical from the beginning of development in these cases to include subgroups of subjects with genetic variants in metabolic or transporter pathways.

Strategies other than a targeted candidate gene approach can also be useful for probing the causes of variability early in drug development, even before there is understanding of the influence of genetic factors on drug response. Strategies include routine retrospective genotyping of subjects in early-phase clinical trials using a gene chip that includes a large number of possible candidate metabolism and transporter genes, some of which may influence PK and/or PD. Such studies may identify the gene variants that contribute to PK or PD variability that were not anticipated based on prior in vitro or clinical data. Exploration of other genetic contributors to PK is indicated when the exposure-response relationship is well-defined, observed drug exposures are variable across subjects, and in vitro data do not implicate specific metabolism pathways or transporters. Because of the multiple hypotheses being tested with this approach, associations can be confirmed by in vitro studies or staged analysis designs (e.g., discovery cohort followed by replication cohort) before considering additional prospective clinical studies.

For a drug that is a substrate of a polymorphic enzyme or transporter, the evaluation of comparative PK in the subgroups genetically defined as UM (ultra-rapid metabolizers), EM (extensive metabolizers), IM (intermediate metabolizers), and PM (poor metabolizers) often provides essential information on potential drug–drug interactions. This is helpful in prioritizing subsequent drug interaction studies and in estimating the extent of interactions by that specific pathway. For example, the difference in drug exposures between EM and PM subgroups would generally represent the most extreme change that could be caused by a strong inhibitor of that pathway. Similarly, an alternative to a genotype-specific PK study in the case of a polymorphic metabolizing enzyme is to administer the investigational drug to extensive metabolizers with and without concomitant administration of a known strong inhibitor of the metabolic pathway. For example, an individual who is a *CYP2D6* EM can be converted to a de facto *CYP2D6* PM by concomitant administration of a strong *CYP2D6* inhibitor. For drug interaction study design and selection of strong inhibitors of metabolic pathways, refer to the FDA guidance for industry on *Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling*.

2.   *PK and PD Studies in Patients*

If important variability in PK of active species (i.e., parent drug and/or its active metabolite) is observed in healthy volunteers, the significance of this finding should be considered in the design of subsequent studies in patients (e.g., in dose/response studies

in genotype-defined subgroups). When specific genotypes are shown to reliably predict blood levels and drug effect, this knowledge can be used in the subsequent design of other clinical trials, for example, by using genotypes to (1) select patients for trials (e.g., enrichment with potential responders and elimination of patients likely to experience toxicity); (2) stratify groups within trials; and (3) adjust doses in trials. These steps can increase the average effect, decrease toxicity, and improve the chances of overall success of the study. The trials can also reveal exposure differences that are not clinically critical, in which case additional PGx studies may be given lower priority.

3. *Dose-Response (D/R) Studies*

D/R studies are usually conducted in phase 2 using biomarkers or clinical endpoints that are relevant to clinical efficacy and safety to (1) provide proof of concept, (2) identify doses for phase 3 trials, and (3) establish dose-response for relatively common adverse effects. Both PK differences (i.e., metabolism and transport) and PD differences (e.g., shift in concentration-response curves) can lead to differences in D/R in individuals. If previous PK and/or PD studies suggest that a genotype or phenotype is important in influencing systemic exposure-response or efficacy and safety responses, D/R studies that stratify dose groups by genotype or specific genotype-guided D/R studies (PK adjusted D/R or even a concentration controlled study) should be considered. In the latter studies, doses are defined by expected blood levels in individuals rather than by administered dose.

Drug blood level evaluation in D/R studies, even if the study was not planned to assign patients to groups by blood levels, can help interpret results when there are major differences in blood levels resulting from genomic factors as well as apparent variability in D/R relationships. Genetic information can be included in D/R or concentration/response (C/R) models much like any other clinical covariate (see section C.3, Multiple Covariate Considerations). Explanations related to genomic factors can sometimes be persuasive on their own or can lead to hypotheses to be tested in further studies, where patients would be stratified by genotype.

C.   Specific Considerations in Study Design

1. *Overview*

The choice of study design depends on prior knowledge and the purpose of the study. The study is straightforward when the goal is to compare PK in genomically defined subgroups of healthy volunteers or patients. Often, PK can be assessed in the relevant subgroups in the same study. This design is similar to studies in people with hepatic or renal impairment. These studies provide information on exposure in genomically defined subgroups and, depending on an understanding of the PD consequences of blood levels (e.g., concentration/response relationships), could influence dosing in later randomized controlled trials. The information and results would generally be included in product labeling.

Less well developed than such PK studies are study designs used to identify genomically distinguished PD responses. When a particular genomic influence is reasonably well understood, patients should be stratified and responses analyzed by subset. This would be possible, for example, for oncology settings when tumor markers (e.g., estrogen receptor status, *EGFR* mutation status, and *KRAS* mutation status) are thought to predict response, and historically when other markers have had similar potential (e.g., high vs. low renin, systolic or diastolic dysfunction in heart failure). In a more exploratory setting, PK-PD studies or D/R studies measuring a biomarker can be examined for genomic predictors of PD effects. Earlier exploratory studies in drug development will generally look for effects on such a marker, while studies of clinical endpoints would be assessed later in drug development. In some cases, of course, there is no marker, and only clinical outcomes can be studied. In later trials, such relationships, if not anticipated, would in most cases be considered exploratory (i.e., needing prospectively defined confirmation).

Analytical validation of genotyping and phenotyping methods should be established before initiating a clinical PGx study. Appropriate quality control materials, standards, and calibrators (where applicable), as well as validated protocols, should be established to provide assurance of continuing analytical performance over time and across testing sites. Analytical validity is critical if the genomic biomarker is intended to select patients for entry into pivotal efficacy studies; if the results of the genomic test are intended to determine whether a patient is to receive the drug, the assay may require approval under an Investigational Device Exemption by CDRH. For use as an in vitro device companion diagnostic, the test must be approved or cleared, except in specified circumstances, at the same time the drug is approved for marketing (see footnote 5). For specific advice on analytical and clinical validity requirements for FDA approval or clearance of a genomic assay, sponsors should consult the Office of In Vitro Diagnostics and Radiological Devices (OIR) in CDRH, especially if co-development of a drug and diagnostic is planned.

2.   *Study Population*

Clinical PGx studies focused on pharmacokinetics are usually performed in phase 1 using healthy volunteers, with additional attention to the effects of gender, age, and race/ethnicity. Safety considerations may preclude the use of healthy volunteers for certain drug classes (e.g., cytotoxic anticancer drugs). Studies of patients for whom the investigational drug is intended provide the opportunity to explore PD or clinical endpoints not measurable in healthy volunteers.

The exclusion of patients with certain genotypes from a clinical trial should be considered on a case-by-case basis, but may be appropriate when the concentration/response relationship is reasonably well known and it is clear that subjects with certain genotypes would experience toxicities or not respond to the low exposure of active drug that would be achieved. Similarly, the clear absence of a drug target (e.g., the gene for a cell surface receptor needed for anticancer activity) might lead to exclusion of such patients. A potential problem, however, may be lack of information as to what

receptor level is needed for response. Many quantitative genomic markers do not lead to "all or none" responses; therefore, inclusion of patients with a range of target levels in early-phase trials is advisable to enable exploration and refinement of optimal diagnostic cut-offs for prospective testing in phase 3 trials.

When a drug–drug interaction study is intended to evaluate the impact of an investigational drug as an inhibitor of enzyme metabolism, it would not be appropriate to enroll poor metabolizers of that enzyme if it is polymorphic. In some instances, an evaluation of the extent of drug interactions in subjects with various genotypes may be helpful (refer to the FDA's guidance for industry on *Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling.*

3.   *Multiple Covariate Considerations*

Many observable phenotypes of drug response in humans result from the interactions of multiple factors or covariates, including genetic, demographic, and environmental factors. The understanding of specific covariates (e.g., age, sex, and race) and gene-covariate interactions on variability in drug response could be useful in understanding the relative impact of genetics, versus other nongenetic factors, on the PK, PD, dosing, efficacy, and safety of a drug. For example, some clinical studies have found that genetic variants in *CYP2C9* and *VKORC1* increase the risk of bleeding in patients taking standard doses of warfarin. However, genetic variations are not the only factors that increase the risk of bleeding. The dose of warfarin in the context of a patient's body surface area (BSA) and age may also influence the bleeding risk. In most cases, therefore, it is important to understand the risk associated with multiple factors — both genetic and nongenetic.

Mathematical simulations using population-based, physiological PK models (i.e., physiologically based pharmacokinetic (PBPK) models) that simultaneously integrate various patient-intrinsic and -extrinsic factors can provide an understanding of the potential complex changes in E/R relationships in patients when multiple covariates are present. Some applications of these models, including the design of clinical trials to evaluate the effects of drug-metabolizing enzyme polymorphisms on PK and PD, are increasingly being seen in regulatory submissions (Zhao et al., 2011).

4.   *Dose Selection*

A clinical PGx study should be conducted at relevant clinical doses. A lower dose or different titration interval can be used in subjects with certain genotypes that could cause high and unsafe exposure or excessive pharmacological response to the drug. Interpretation of findings in a clinical PGx study, such as changes in exposure in specific genotypes, may be aided by a good understanding of dose- or concentration-response relationships for both desirable and undesirable drug effects in the general population and in subpopulations with different genetic variations. FDA's guidance for industry on *Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications* discusses considerations in the evaluation of D/R and concentration/response (C/R) relationships.

5.   Measurements of Interest

- PK Parameters

PK measurements and parameters that should be useful for consideration of genotypic effects on drug exposure include AUC, C_{max}, and time-to-C_{max} (T_{max}), as well as other PK parameters such as clearance, volumes of distribution, and half-lives. Additional measures, such as trough drug concentrations in multiple-dose PK studies (if associated with a PD measurement, an efficacy endpoint, or an adverse effect) can help determine appropriate dosing strategies to achieve similar exposure across different subsets of the population.

- Biomarkers of Drug Response (PD)

Biomarkers of drug response related to a drug's intended pharmacological effect, suspected off-target effects, and/or safety, when available, should be incorporated into clinical PGx studies to measure whether or not genetic factors influencing exposure or target response will have an impact on clinical outcomes.

6.   Statistical Considerations

Clinical studies evaluating the effect of PGx factors during early drug development are intended to address questions concerning variability in PK and short-term PD endpoints in healthy volunteers and patient groups. Although there are well-established variants in metabolism (e.g., *CYP2D6* and *CYP2C19* poor metabolizers), most pharmacogenomic research is exploratory at this stage and is often intended to discover relationships for which no prior hypotheses exist. Even when genomic factors are expected to be important (e.g., genetic determinants of receptor characteristics), there may be considerable uncertainty as to clinical consequences.

Statistical considerations in PGx studies are important. The hypotheses and conclusions arising from early-phase clinical studies (e.g., need for different doses for different CYP genotypes) should be sufficiently supported with credible data. When not definitive, those data should define a hypothesis to be pursued later in drug development with more rigorous study designs. Considering the limited number of subjects within or across early-phase clinical pharmacology studies, such studies may be able to identify only large differences in PK resulting from genomic differences (e.g., *CYP2D6* poor metabolizers) that are likely to be clinically relevant; genomic PD differences will generally need further study.

For exploratory and observational studies, which generally do not involve randomization of subjects to treatment, the statistical concepts that are most relevant to clinical pharmacogenomics include the following:

1. Avoid confounding by balancing the testing of samples on all controllable confounding factors, which include technical variations (e.g., day, operator,

instrument, reagent lot, sampling handling/storage/preparation/dilution) and patient variations (e.g., outcome, disease stage, treatments, co-morbidities, other medical history, demographics, clinical covariates). This can be accomplished by employing experimental design principles such as replication, blocking, randomization (e.g., of sample test order), and masking/blinding (e.g., of assay operator to outcome, and, conversely, of outcome evaluator to assay result).

2. Control for the multiplicity and the risk of incorrectly identifying associations in genomic data when many searches are performed (e.g., using Bonferroni correction, false discovery rate, or other method).

3. Quantitatively characterize the preliminary marker classifier performance (e.g., sensitivity, specificity, predictive values, reclassification, or other metrics) and explore the prognostic and predictive attributes of the marker when appropriate.

4. Address the potential for population stratification due to admixture and other confounding factors (i.e., factors that are responsible for the effect and that are associated with a genetic difference).

5. Assess the reproducibility of a genetic finding (strength of association, magnitude of association, repeatability in multiple datasets, subgroup response) so that it can be used reliably and evaluated in follow-up development in later-phase clinical trials.

Highly multiplexed genotyping methods are available, for instance, to interrogate hundreds or thousands of sequence variations in ADME-related genes. These methods may be useful when exploring causes for PK variability in the absence of in vitro data that suggest a single causal pathway. Early-phase studies are often not adequately powered to define PGx relationships when only a few subjects with a variant genotype may have been enrolled. Consequently, pooled analysis of clinical pharmacology studies with similar protocols enables more comprehensive and reliable investigations of multiple genetic factors while retaining the ability to control for false positive results. The correlative analysis methods employed in such studies are similar to the pharmacometric methods used to evaluate the effects of age, race, and other clinical covariates on drug exposure. Considering the exploratory nature of these investigations, replication of the findings or experimental studies (e.g., follow-up in vitro studies) may be recommended to confirm clinical relationships between a genetic factor and drug exposure. Typical methods to filter genetic markers for association testing should be applied (e.g., Hardy-Weinberg equilibrium deviations).

In PGx substudies that are not randomized, it is possible for the substudy population to be different from the overall trial population in a variety of ways. If a trial is randomized and the substudy is selected based on a baseline feature, the groups in the randomized sample, if they are a reasonably large fraction of the sample, should be sufficiently similar to allow for meaningful assessment. In all cases, however, a between-group difference could be the result of the genetic difference or of some characteristic related to the genetic difference. The statistical analysis of genetic substudies should include an assessment of differences in clinical or demographic characteristics and an assessment of heterogeneity in treatment effects between the substudy and overall trial populations. When incomplete sampling is related to geographic or site differences in participation,

treatment effects at the participating sites should also be estimated. Power analysis should be performed to determine what effect sizes can feasibly be identified in a subset of the trial, including the power to detect genotype by treatment interactions.

Statistical issues for early pharmacogenomic assessments can be divided roughly into the following three categories according to the use of the PGx information:

1. To define differences in metabolism or clearance that will affect the PK of a drug

When a genetically polymorphic pathway has an important role in the drug's PK, the magnitude of the variability in PK parameters according to genotype should be examined. In some cases, the effect of the altered PK will confer an important effect on PD or clinical endpoints, depending on the C/R relationship. Studies will initially simply compare PK in groups with normal and gene-variant metabolism, and often the differences will be large and readily described. If the clinical consequences of the genetic differences need to be investigated, studies in patients with normal and genetic-variant profiles should be studied (in separate studies, or preferably as strata within a single study), and the differences in response between the two groups should be noted.

2. To define differences in the magnitude or presence of a favorable response to a treatment

When genetic differences do not lead to differences in blood levels of active drug or metabolite, but rather to changes in the C/R relationship (e.g., slope in C/R curve, or at the extreme, lack of any PD effect), validation and precise definition of the genomic difference should be evaluated in a trial in which genetic strata can be studied, preferably in the same trial. Thus, cancer patients whose tumors bear a particular genetic marker and patients without the marker would be stratified in a controlled trial and differences in response between the groups studied.

A critical question is how such markers would be identified initially as an a priori hypothesis. If not understood as a mechanism of drug action initially, they should be identified later, after studying the exposure of an unselected population to the drug. Response could then be explored in relation to a wide range of markers, possibly with samples from related studies. Considering the small sample size available in premarket clinical databases, exploratory studies such as genome-wide association studies can be powered to detect only large genetic effects. Even if a strong association were seen, it would generally be necessary to conduct a focused examination to replicate the relationship in recognition of the possibility of false findings from multiple testing and bias within the initial dataset and the typically small sample sizes at this stage. A plan for an interim evaluation, perhaps of a critical biomarker, could provide early confirmation if the effect were in fact large.

In addition, if use of the test result for the purpose of selecting patients or dosing regimens for clinical trials leads to labeling that directs treatment to a genomically defined patient subset, the test would ordinarily require CDRH approval.

3. To identify genomic predictors of an increased likelihood of an adverse effect

Relating a PGx characteristic to adverse drug reactions (ADRs) requires, first, analysis of patients who experience the ADR to seek a common PGx characteristic. This would generally involve a case-control approach, comparing the frequency of the putative PGx predictor in patients with and without the adverse reaction. Given the generally small sample sizes and substantial multiplicity in such searches, a very large genetic effect on risk for the adverse event and clear statistical significance for the association would generally be needed to be persuasive. In many cases, it may not be possible to characterize PGx relationships for relatively rare or severe adverse reactions in the premarket setting. High baseline sample acquisition rates and comprehensive follow-up data may, however, allow for better characterization of the relationship between a genetic variant and risk for adverse reactions. As a general matter, in addition to high sample acquisition rates, a clinically meaningful definition of cases and clearly described controls would be important. Alternative definitions of cases and controls may be useful as sensitivity analyses (e.g., using different thresholds for biochemical laboratory values to define drug-induced organ injury). The ultimate usefulness of the data will depend on aspects of the results that include, but might not be limited to, the strength of the association, consistency of the findings across subgroups (e.g., race groups/countries), and relevance to the U.S. population, as well as the intention for the data's use (e.g., removal of a clinical hold, modified labeling).

V.   INCLUDING PHARMACOGENOMIC INFORMATION IN LABELING

In general, labeling should include information on PGx only if it is useful to inform prescribers about the impact (or lack of impact) of genotype on phenotype; or to indicate whether a genomic test is available, and, if so, to indicate whether testing should be considered, is recommended, or is necessary. If applicable, a "Pharmacogenomics" subsection should be included in the CLINICAL PHARMACOLOGY section (e.g., as "12.5 *Pharmacogenomics*") of the prescribing information (PI) and should include clinically relevant data or information on the effect of genetic variations affecting drug therapy.

In labeling, pharmacogenomic information can include, but is not limited to, the following:

*   Information on the frequencies of alleles, genotypes, haplotypes, or other genomic markers of relevance
*   Description of the functional effects of genomic variants (e.g., genetically based differences in enzyme activity such as reduced cytochrome P450 enzyme activity attributable to polymorphisms in a CYP gene)
*   Effect of genotype on important PK parameters or PD endpoints
*   Description of pharmacogenomic studies that provided evidence of genetically-based differences in drug benefit or risk
*   Dosing and patient selection recommendations based on genotype

When the information has important implications for the safe and effective use of a drug and the consequences of the genetic variations result in recommendations for restricted use, dosage adjustments, contraindications, or warnings, this information will be summarized in other sections of the labeling, as appropriate (e.g., BOXED WARNING, INDICATIONS AND USAGE, DOSAGE AND ADMINISTRATION, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, DRUG INTERACTIONS). A cross-reference to the more detailed data supporting the clinical recommendation (typically in CLINICAL PHARMACOLOGY or CLINICAL STUDIES) should be included. If there is PGx information that is important to convey to patients, it should be summarized in the PATIENT COUNSELING INFORMATION section (17).

Detailed information about clinically relevant genetic information should be consolidated into the most appropriate labeling section. The following chart shows types of PGx information that could appear in various sections of labeling.[6]

Section of Label	Types of Information
INDICATIONS AND USAGE	PGx information related to proper patient selection (e.g., the need for PGx testing)
DOSAGE AND ADMINISTRATION	Dosing recommendations for subgroups of patients based on genetic makeup
BOXED WARNING, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, and/or ADVERSE REACTIONS	PGx information affecting drug safety
WARNINGS AND PRECAUTIONS and USE IN SPECIFIC POPULATIONS	Genotype(s) that are known to be associated with an adverse reaction in a specific population
DRUG INTERACTIONS	Relevant information concerning the role of genetic variations in drug-drug interactions, and the clinical consequences of the combination of genetic polymorphisms in protein(s) in the context of the drug's metabolism, transport, and action
CLINICAL PHARMACOLOGY	PGx impact on PK or PD
CLINICAL STUDIES (if studied and the evidence is substantial; or if observed neutral findings (i.e., lack of a pharmacogenetic effect) would be pertinent clinical information)	Efficacy differences related to PGx

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[6] FDA has issued the draft guidance for industry *Labeling for Human Prescription Drug and Biological Products — Implementing the New Content and Format Requirements*. Once finalized, this guidance will represent the Agency's thinking on this topic.

REFERENCES

FDA Guidances[7]

Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling (Draft)

E15 *Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories* (ICH guideline)

Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications.

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[7] FDA guidances, including ICH guidances, are available on FDA's guidance Web page. We update guidances periodically, and draft guidances will be finalized. To make sure you have the most recent version of a guidance document, check FDA's guidance Web site at FDA Basics for Industry > Guidances. (http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234622.htm).

APPENDIX — Examples of the Value of Pharmacogenomics

PGx information obtained from genomic investigations during the course of drug development can inform drug development and enhance the effectiveness and safety of drugs. Several postapproval examples have illustrated the value of pharmacogenomic discoveries in understanding variable dose requirements (e.g., warfarin (*VKORC1*/*CYP2C9*), tetrabenazine (*CYP2D6*), pimozide (*CYP2D6*)), failure of intended pharmacological effect (e.g., clopidogrel (*CYP2C19*), cetuximab and panitumumab (KRAS)), and adverse reaction predisposition (e.g., abacavir (*HLA-B*), carbamazepine (*HLA-B*)).[8]

These examples illustrate principles that, if considered early in drug discovery and development, can (1) provide experimental evidence for a pharmacogenomic interaction (or, importantly, evidence that genetic variation is actually not clinically relevant); (2) define the relevance of polymorphic pathways to drug metabolism and pharmacokinetics; (3) help determine whether entry criteria for dose-finding studies should be modified to include or exclude certain subsets of patients; (4) identify important stratification and enrichment factors for clinical trials across phases of drug development; and (5) aid in development of instructive labeling for therapeutic products under review for regulatory approval.

Abacavir (Ziagen): Improving the Benefit–Risk (B/R) Relationship by Identifying Patients at High Risk for a Serious Adverse Reaction

> Abacavir, which is used alone or in combination with other drugs, is an antiretroviral drug used in the treatment of HIV-1 infection. An abacavir hypersensitivity reaction (HSR) was observed in about 5 to 8% of clinical trial patients, so that hypersensitivity was a well-recognized problem at the time of approval. The clinical manifestations of the HSR included fever and/or rash and, to a lesser degree, gastrointestinal symptoms (nausea, vomiting, diarrhea, and stomach pain) and/or respiratory symptoms (cough, shortness of breath, and sore throat) that emerged within the first 6 weeks of treatment in more than 90% of patients with HSR. Symptomatology worsened with continued therapy and could be life-threatening, but usually resolved upon discontinuation of the drug. Clinical diagnosis was imprecise because of the patients' concurrent illness or drug treatments, and there was an HSR rate of 2 to 3% in the standard of care arm without abacavir in blinded clinical trials. The hypersensitivity reactions were an important limitation to the use of abacavir.
>
> Approximately 3 to 4 years after marketing approval of abacavir, new PGx research identified an allele (*HLA-B**5701) that appeared to be associated with the hypersensitivity reactions, but the sensitivity and specificity of this predictor of HSR varied between studies and racial populations. A 6-week, randomized controlled trial (called PREDICT-1) (Mallal S et al., 2008) was undertaken to assess the clinical utility of

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[8] The examples provided illustrate historical approaches to updating the labels of drugs. Currently, FDA expects that if a diagnostic test is essential for the safe and effective use of a therapeutic product (i.e., a companion diagnostic), there should be a cleared/approved test with the appropriate intended use available concurrent with the drug label change.

HLA-B*5701 screening before beginning abacavir treatment. Abacavir-naïve patients (n = 1956) were randomized 1:1 to an abacavir-containing regimen with HSR monitoring according to standard of care (control arm), or to an abacavir-containing regimen with HSR monitoring preceded by prospective *HLA-B**5701 screening (PGx arm). In the PGx arm, patients who tested positive for *HLA-B**5701 were excluded, and only *HLA-B**5701 negative patients were enrolled.

The trial had two co-primary endpoints: the rate of clinically suspected HSR, and the rate of immunologically confirmed HSR, defined as HSR with a positive patch test reaction. The incidence of clinically suspected HSR was 7.8% and 3.4% in the control and PGx arms respectively (P<0.001). The positive predictive value of the *HLA-B**5701 test for clinically suspected HSR was 61.2% and the negative predictive value was 95.5%. For immunologically confirmed HSR, the rate was 2.7% for the control arm and 0% for the PGx arm (P<0.001). The positive predictive value of the *HLA-B**5701 test for immunologically confirmed HSR was 47.9% and the negative predictive value was 100%, that is, about half of the patients with a positive *HLA-B**5701 test developed confirmed HSR while no patient with a negative *HLA-B**5701 test did. These findings illustrate the importance of a precise definition of the event that is to be predicted by the genetic marker (i.e., phenotype definition).

The impact of the PREDICT-1 results was substantial because the study was a prospectively planned randomized trial with essentially full ascertainment of genomic status of every randomized subject. The study provided demonstration of clinical usefulness (i.e., near total ability to avoid abacavir-induced HSR with an acceptable false positives rate in the screening). The results of this study influenced the inclusion of strong recommendations for *HLA-B**5701 screening in professional guidelines and in the approved labeling (updated July 2008).

Clopidogrel (Plavix): Identifying Patients with Reduced Response to a Drug

Clopidogrel is a platelet adenosine diphosphate (ADP)-receptor antagonist that is indicated for reduction of atherothrombotic events in patients with recent myocardial infarction, recent stroke, peripheral artery disease, and acute coronary syndrome. Clopidogrel is a prodrug with no antiplatelet activity, but about 15% of the dose is metabolized to an active metabolite in a two-step process involving multiple CYP enzymes, one of which is the polymorphic *CYP2C19*. *CYP2C19* has four different metabolizer phenotypes: ultrarapid, extensive, intermediate, and poor.

Analyses of data from several PK/PD studies demonstrated that carriers of at least one loss-of-function (LOF) allele of *CYP2C19* showed reduced exposure to the active metabolite and less inhibition of platelet aggregation (Mega et al., 2009). These observations have been independently replicated in numerous clinical pharmacology studies. The relationship between the *CYP2C19* genotype and PK/PD was further extended to clinical outcomes in several population- or clinical trial-based cohort studies (Mega et al., 2010). For example, *CYP2C19* genotypes were determined in patients enrolled in the TRITON-TIMI 38 trial who voluntarily consented to provide their blood

samples for DNA analysis. In TRITON-TIMI 38, carriers of LOF *CYP2C19* alleles had a higher rate of death, nonfatal myocardial infarction, or nonfatal stroke than non-carriers (no LOF allele) following percutaneous coronary intervention. In addition, the rate of stent thrombosis over the same time period was approximately three times higher in carriers.

Based on the results from multiple clinical pharmacology and outcome studies, the label of clopidogrel was updated in May 2009 and again in March 2010 to include PGx information related to the diminished antiplatelet responses and the increased risk of cardiovascular events in patients with reduced *CYP2C19* function. Many publications have replicated the association between *CYP2C19* variants and clopidogrel responsiveness since the last labeling update. Collection of DNA in phase 3 trials helped substantiate the findings of several clinical pharmacology studies.

Warfarin (Coumadin): Selecting Optimal Doses Based on Genotype-Based Differences in PK and/or PD

Warfarin is a coumarin-based anticoagulant that is widely used for the short- and long-term management of thromboembolic disorders, such as deep-vein thrombosis, and to prevent stroke and systemic embolic events in patients with atrial fibrillation and those undergoing orthopedic surgeries. A relatively large number of patients experience life-threatening bleeding complications from warfarin. It has been consistently a top ten-ranked cause of drug-induced serious adverse reactions. Major bleeding episodes are reported relatively frequently. However, it is also essential to achieve adequate anticoagulation to prevent thromboembolic events that warfarin is intended to prevent.

Warfarin has a narrow therapeutic range, with wide variation in dose requirements for individual patients, and dose is modified by testing of INR (International Normalized Ratio), a measure of coagulation inhibition. Titrating warfarin-naïve patients to a stable INR range (e.g., 2-3, sufficient but not excessive anticoagulation) in a reasonable time is a significant challenge for health care professionals because of the many genetic (e.g., *CYP2C9* and *VKORC1*) and nongenetic (e.g., sex, body size, drug-drug interactions, diet) factors affecting the PK and PD of warfarin. Underlying genetic factors have been shown to account for approximately 35-40% of the variation in the maintenance dose. *CYP2C9* is the hepatic enzyme responsible for metabolizing S-warfarin, which is 3-5 times more potent than the R-enantiomer. Genetic polymorphisms affecting *CYP2C9* (e.g., the *2 and *3 alleles) are common in the general population, resulting in decreased clearance and higher blood levels of S-warfarin.

Warfarin works by inhibiting vitamin K epoxide reductase (VKOR), which is encoded by the *VKORC1* gene. Polymorphisms in this gene affect an individual's response to warfarin. Studies have shown that *CYP2C9* and/or *VKORC1* genotypes play an important role in determining the dose of warfarin. In August 2007, FDA updated the warfarin labeling to provide general information about genetic testing and to encourage health care professionals to use this information for initial dosing to reduce the risk of both bleeding and undertreatment. Subsequently, in January 2010, the warfarin labeling

was updated to include a dosing table to be used for dosing when *CYP2C9* and *VKORC1* genotype information for a patient is known.