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The role of ADME pharmacogenomics in early clinical trials: perspective of the Industry Pharmacogenomics Working Group (I-PWG)

Genetic polymorphisms in metabolizing enzymes and drug transporters have been shown to significantly impact the exposure of drugs having a high dependence on a single mechanism for their absorption, distribution or clearance, such that genotyping can lead to actionable steps in disease treatment. Recently, global regulatory agencies have provided guidance for assessment of pharmacogenomics during early stages of drug development, both in the form of formal guidance and perspectives published in scientific journals. The Industry Pharmacogenomics Working Group (I-PWG), conducted a survey among member companies to assess the practices relating to absorption, distribution, metabolism, excretion pharmacogenomics during early stages of clinical development, to assess the impact of the recent Regulatory Guidance issued by the US FDA and EMA on Industry practices.

Keywords: absorption, distribution, metabolism and excretion • ADME • drug metabolism • drug transporters • pharmacogenomics • pharmacokinetics • Regulatory Guidance

PGx impacting PK variability

Understanding the intrinsic and extrinsic factors underlying variability in exposure between individuals is a key objective during the early stages of clinical drug development [1]. Within a clinical trial population there is often a strong correlation between exposure and response (pharmacokinetic–pharmacodynamic [PK–PD] relationship) [2]. Defining the major factors of intersubject variability can improve both the efficacy and safety of a drug by controlling the relevant extrinsic factors (e.g., drug interactions with concomitant drug administration) or potentially adjusting dose in consideration of intrinsic factors [3]. Genetic polymorphisms in metabolizing enzymes and drug transporters have been shown to significantly impact the exposure of drugs having a high dependence on a single mechanism for their absorption, distribution, clearance (metabolism, renal or biliary excretion) or kinetics (formation or clearance) of an active metabolite (absorption, distribution, metabolism, excretion [ADME]). Therefore,

genotyping can lead to actionable steps (drug or dose selection, **Table 1**) in disease treatment across a wide range of therapeutic areas, including depression [4], inflammatory bowel disease [5], atherosclerosis [6] and inflammation [7]. Consequently, pharmaceutical companies, academic scientists and regulatory agencies have increasingly studied and provided information on developmental compounds and marketed drugs with respect to pharmacogenetics of enzymes and transport proteins [8,9]. While this Perspective focuses mostly on the drug metabolizing and drug transporter genes there are additional classes of polymorphic genes which also play a role in drug efficacy and safety, such as VKORC1.

Role of regulators in drug development

Understanding how to appropriately apply areas of evolving science in the drug development process can best be achieved through collaborative input from scientists in industry, academia and regulatory agencies. Meeting symposia, publications and

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Table 1. A list of absorption, distribution, metabolism, excretion biomarkers with the associated drug-gene pairs from publicly available databases.

HUGO symbol	# of entry	Drug listed [†]
CYB5R1–4	1	Metoclopramide [‡]
CYP1A2	1	Dexlansoprazole [‡]
CYP2C19	15	Amitriptyline [‡] , Carisoprodol [‡] , Citalopram ^{‡,§,¶} , Clobazam [‡] , Clomiprine [§] , Clopidogrel ^{‡,§,¶} , Dexlansoprazole [‡] , Diazepam [‡] , Doxepin [§] , Drospirenone [‡] and Ethinyl [‡] , Estradiol [‡] , escitalopram [¶] , Esomeprazole ^{‡,¶} , Imipramine ^{‡,§,¶} , Lansoprazole ^{‡,¶} , Moclobemide [¶] , Omeprazole ^{‡,¶} , Pantoprazole ^{‡,¶} , Prasugrel [‡] , Rabeprazole [‡] , Ticagrelor [‡] , Trimipramine [§] , Voriconazole ^{‡,¶}
CYP2C9	3	Acenocumarol [¶] , Celecoxib [‡] , Flurbiprofen [‡] , glibenclamide [¶] , Glimepiride [¶] , Glimepiride [¶] , phenprocoumon [¶] , Phenytoin [¶] , Rabeprazole [¶] , Tolbutamide [¶] , Warfarin ^{‡,§}
CYP2D6	37	Amitriptyline ^{‡,§,¶} , Aripiprazole ^{‡,¶} , Atomoxetine ^{‡,¶} , Carvedilol ^{‡,¶} , Cevimeline [‡] , Citalopram [‡] , Clomipramine ^{‡,§,¶} , Clozapine ^{‡,¶} , Codeine ^{‡,§,¶} , Desipramine ^{‡,§} , Dextromethorphan [‡] , Doxepin ^{‡,§,¶} , duloxetine [¶] , flecainide [¶] , Flupenthixol [¶] , Fluoxetine [‡] , Fluvoxamine [‡] , Galantamine [‡] , haloperidol [¶] , lloperidone [‡] , Imipramine ^{‡,§,¶} , Metoprolol ^{‡,¶} , Mirtazapine [¶] , Modafinil [‡] , Nefazodone [‡] , Nortriptyline ^{‡,§,¶} , Olanzapine [¶] , Oxycodone ^{§,¶} , Paroxetine ^{‡,¶} , Perphenazine [‡] , Pimozide [‡] , Propafenone ^{‡,¶} , Propranolol [‡] , Protriptyline [‡] , Quinidine [‡] , Risperidone ^{‡,¶} , Sertraline [¶] , Tamoxifen [¶] , Terbinafine [‡] , Tetrabenazine [‡] , Thioridazine [‡] , Tolterodine [‡] , Tramadol ^{‡,¶} , Trimipramine ^{‡,§} , Venlafaxine ^{‡,¶} , Vortioxetine [‡] , Zuclopenthixol [¶]
CYP3A5	0	Tacrolimus [¶]
DPYD	2	Capecitabine ^{‡,¶} , Fluorouracil ^{‡,§,¶} , Tegafur ^{§,¶}
G6PD	19	Chloroquine [‡] , Chlorpropamide [‡] , Dabrafenib [‡] , Dapsone [‡] , Glimepiride [‡] , Glipizide [‡] , Glyburide [‡] , Mafenide [‡] , Methylene Blue [‡] , Nalidixic acid [‡] , Nitrofurantoin, PEG-(Sodium sulfate, Sodium chloride, Potassium chloride, Sodium ascorbate, and Ascorbic acid) [‡] , Pegloticase [‡] , Primaquine [‡] , Quinine sulfate [‡] , Rasburicase ^{‡,¶} , Sodium nitrite [‡] , Succimer [‡] , Sulfamethoxazole and Trimethoprim [‡]
NAT1–2	2	Isosorbide and Hydralazine [‡] , Rifampin (Isoniazid, and Pyrazinamide) [‡]
TPMT	4	Azathioprine ^{‡,§,¶} , Cisplatin [‡] , Mercaptopurine ^{‡,§,¶} , Thioguanine ^{‡,§,¶}
UGT1A1	4	Indacaterol [‡] , Irinotecan ^{‡,¶} , Nilotinib [‡] , Pazopanib [‡]
VKORC1	1	Acenocumarol [¶] , Warfarin ^{‡,§}
SLCO1B1	0	Simvastatin [§]
Total	90	

[†]Based on publicly available information as of 18 August 2014.

[‡]US FDA Pharmacogenomics table.

[§]Dosing guidelines which take into consideration patient genotype and have been published by the Clinical Pharmacogenetics Implementation Consortium.

[¶]Dosing guidelines which take into consideration patient genotype and have been published by the Royal Dutch Association for the Advancement of Pharmacy – Pharmacogenetics Working Group.

public hearings all have served as forums for sharing and discussing scientific advances and perspectives on ADME PGx [10,11]. Initially, regulatory influence was seen in the review and in some instances, revision of Product Labels for approved drugs (e.g., 6-mercaptopurine, irinotecan, warfarin and clopidogrel [10]), and more systematically via regulatory guidance. Examples of guidance relating to information required for new drug submissions were specified in 2005 [12]. Since then the number of approved drugs with ADME PGx

labeling information have substantially increased [13]. In tandem with this, drug labels have become increasingly consistent with polymorphic drug metabolizing enzyme and drug transporter drug–drug interaction guidance [14]. More recently, regulatory agencies in the USA, EU and Japan have provided greater guidance for assessment of PGx during early stages of drug development, both in the form of formal guidance and perspectives published in scientific journals [13,15–16]. An advantage of assessing the potential influence of

ADME PGx on exposure variability in early clinical trials is that those studies typically provide the most complete assessment of pharmacokinetics and control of various extrinsic factors (e.g., subject age, gender, health and exclusion of concomitant drugs).

Survey of current industry practices

The I-PWG, a working group open to any company within the pharmaceutical industry and presently comprised of 21 member companies [17], recently conducted two surveys among member companies to assess the practices relating to ADME PGx in drug development, both preclinically (In Press, W Brian, et. al., *Pharmacogenomics*, 2015) and during early stages of clinical development (Phase 1–2). The I-PWG (International Pharmacogenomics Working Group) clinical survey was completed by 17 member companies in the fall of 2013. Company size, as judged by yearly R&D (research and development) budgets during the past fiscal year, included five companies with less than US\$1 billion in budget, two companies in the \$1–2 billion range and nine companies with more than \$2 billion in R&D budgets. One member company chose not to answer the question. This article does not necessarily reflect the views of the companies that are members of the I-PWG.

The intent of this survey was to assess the impact of the recent Regulatory Guidance issued by the US FDA and EMA on pharmaceutical industry practices. This article reports the findings of the I-PWG survey regarding clinical practices and provides comparisons to similar surveys conducted in 2006 and 2009 and published in 2008 [18] and 2011 [19], respectively, to assess how advances in the scientific landscape and greater regulatory guidance have changed the use of PGx in early stage of drug development in the ensuing 7 years. We also summarize what we view are current challenges and the future direction for PGx research relating to understanding PK variability.

DNA sample collection & storage

The EMA, FDA and PMDA (Pharmaceutical and Medical Devices Agency, Japan) all strongly encourage prospective collection and storage of DNA samples from all participants in clinical trials [15]. The majority of companies always or usually collect DNA for ADME-related studies in Phase 1 studies Q1 (Question 1), but less frequently in patient trials (Phase 2 and 3) as shown in [Figure 1](#). Compared with 2006 and 2009, a higher percentage of companies always collected DNA samples in all FIH (first in human) studies (33% vs 14 and 23%, respectively) for ADME genotyping. The percentage of companies who always collect DNA from drug–drug interaction studies rose

from 7 to 27%, and an increased number of companies always collected DNA samples from special population studies (19% vs none in 2006), proof of concept (25% vs none), dose ranging (19% vs none) and pivotal (12% vs none and 14%, respectively). Due to lack of specific questions some numbers are absent for the 2009 survey conducted by the I-PWG. For Phase 2 trials, 28% of the companies had a mandatory DNA collection in some trials and 14% for Phase 3 (pivotal) trials. In all, these results point to an increased awareness among member companies of the utility of collecting DNA samples from clinical trials since the 2006 survey was completed but no major changes since 2009. Collecting and banking DNA samples for future PGx research beyond the initial period of the clinical trial was already a standard among companies by 2006 and all companies continued this practice when samples were collected (Q15). Sixteen out of 17 companies who store samples have used them to address emerging issues during or after a clinical trial completion (Q16). The reported purposes for these retrospective analyses will be discussed later in this paper. While increased DNA storage across different clinical trials was reported, a significant percentage (75%, $n = 12$) of companies do not routinely specify ADME PGx analysis in study protocols (Q2). This discrepancy between regulatory expectation and Industry practice in clinical development may spark more discussions between Industry and regulatory authorities on the strategic needs to collect samples. Forty-three percent of our members reported requests or suggestions for sample collections during regulatory review meetings (Q27), although from the data we cannot conclude in what percentage this was actually then implemented.

Pharmacogenomic testing during early clinical trials

The EMA guidance is most specific in its guidance on genotyping, requiring testing in the FIH and subsequent Phase 1 studies if a drug's clearance is suspected to be via a single polymorphic enzyme (>50% based on preclinical data or >25% based on *in vivo* information). Two-thirds of the companies agree with the EMA guidance thresholds to incorporate PGx testing (Q5). However, in practice, this is not reflected in either planning or widespread testing during Phase 1. Only four companies indicated it was standard practice to broadly genotype and collect data across the early programs (Q4), while three companies indicated that specified ADME PGx analysis is always or usually specified in written plan or strategy for the compound (Q6) or the study protocols (Q2).

It appears that the majority of development candidates are not thought to meet that criterion, as 94% of

Figure 1. Frequency of DNA collection with consent for ADME-related genotyping for various study types during clinical development.

the companies indicated a trigger for genotyping (Q4) was when preclinical data indicated the role of a specific polymorphic enzyme in the compound's PK (Pharmacokinetics). Seventy-five percent of the companies would trigger retrospective genotyping when high PK variability was seen in a clinical trial or there was an observation of a PK outlier, consistent with the guidance (Q4).

Companies were queried regarding the frequency of ADME genotyping for specific types of Phase 1 and Phase 2 studies (Q7). During first in human, multiple dose and drug–drug interaction studies, three to five companies always or usually genotyped subjects. Only one company indicated a similarly high frequency of testing in special population or other clinical pharmacology studies, and no company had such a practice in Phase 2. Therefore, the majority of companies include genotyping in studies based on data-driven decisions (Q4,) including preclinical information, but this results in a low frequency of testing during early stages of clinical development.

Clinical study design

Member companies were asked whether ADME genotyping was used for inclusion (Yes – 75%), exclusion (Yes – 69%) or dose-adjustment (Yes – 27%) in the study design (Q8). Incorporation of prospective genotyping for these reasons occurred most frequently for

drug–drug interaction (DDI) (55%) and multiple dose (MD) (46%) studies (Q9). When ADME PGx research has been included in a trial, 9 of 16 companies have conducted studies where subject participation required DNA collection during Phase 1 (56%) and drug interaction studies (53%). Fewer companies have incorporated study designs (Q18) with mandatory participation during Phase 2 studies (33%) or Phase 3 and 4 studies (15% each). Optional participation seems to be used within different clinical phases more frequently (67–100%) among all companies.

ADME pharmacogenomic assay considerations

The regulatory guidelines do not provide a specific list of ADME genes or specific SNPs having functional polymorphisms. In fact, the ADME PGx biomarkers listed in the FDA's Table of Pharmacogenomic Biomarkers in Drug Labeling are not limited to those with functional deficiencies or expression changes. As of 18 August 2014, a total of 161 entries were listed in the table. Of those, 90 entries are related to ADME genes [20]. However, those 90 entries are comprised of only 11 genes, and none of those are transporter genes, with *CYP2D6* having the highest number of entries (Table 1). The EMA acknowledge in their Guideline [16] that *CYP2D6*, *CYP2C19* and *CYP2C9* are well known polymorphic

cytochrome P450 enzymes and Phase II enzymes, UDP-glucuronosyltransferases, N-acetyltransferase-2 and some methyltransferases are also known to play a role in the interindividual variability in PK. While the guideline did not name specific transporters, it did state that the additional contribution of polymorphism in drug transporters has recently been recognized. Additionally, there are currently few guidelines linking the results of PGx tests to specific therapeutic recommendations. At the time the survey was completed (October 2013) the CPIC (Clinical Pharmacogenetics Implementation Consortium) had provided guidelines for nine drug-gene pairs (and four more in draft form), which included five ADME genes (*CYP2C9*, *CYP2C19*, *CYP2D6*, *SLCO1B1*, *TPMT*) and *VKORC1* based on the availability of strong supporting evidence for genetic variations that affect a drug's efficacy and/or safety profile [21]. In February of 2015 the CPIC had established guidelines for an additional 15 drug-gene pairs, bringing the total up to 24 drug-gene pairs (Table 1) in a little over a year and a half [9]. Similarly, the Royal Dutch Association for the Advancement of Pharmacy established the Pharmacogenetics Working Group to provide pharmacogenetics-based therapeutic (dose) recommendations, and currently has categorized 63 unique genes and 132 unique drugs with varying evidence of clinical significance. Those with treatment guidelines relating to ADME genes are also designated in Table 1.

Regarding the ADME genes assayed during clinical trials, the majority of the companies responding to this question currently genotype for *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *UGT1A1*, and *SLCO1B1* gene variants (Q3) (Figure 2). The metabolic trend of compounds currently in development is consistent with the metabolism of prescription drugs as reported by Zanger *et al.* [22], where *CYP3A4/5*, *CYP2C9*, *CYP2C19* and *CYP2D6* metabolize approximately 80% of 156 prescription drugs cleared by liver in US. Compared with the survey in 2006 (Figure 3), fewer companies genotype *CYP1A2* and *MDR1/ABCB1* genes but more test for the transporter gene *SLCO1B1*. Since the last survey there has been increasing evidence for functionally relevant variants in drug transporters, particularly *SLCO1B1*, that can impact drug disposition. This increased awareness based on scientific advances has driven this increased impetus to understand the genetic variability in transporters and the impact on PK variability in general [23–25].

New *CYP3A4* polymorphism

While there is rapidly emerging information on the genetics, epigenetics and regulation of *CYP3A4* [22], additional confirmatory studies are needed to guide companies as to an appropriate set of measures in

clinical studies. Recent finding of an intronic SNP rs35599367, nominated as *CYP3A4**22, which has been associated with lower expression levels of the *CYP3A4* gene, has focused attention on the possible modulation of *CYP3A4* by genetic factors that would have clinical significance [26]. The minor allele frequency of this variant has been reported as being between 5–8% in Caucasians [26]. Should future research substantiate the findings from recent publications [27,28] of a haplotype which clearly segregates individuals by exposure, genotyping during clinical trials for *CYP3A4* could dramatically increase.

Assay platform & quality considerations for ADME genotyping

Currently, there is no specific regulatory guidance on the platforms or quality considerations for ADME genotyping in clinical trials, although quality considerations may be covered by other regulatory mechanisms such as CLIA (Clinical Laboratory Improvement Amendments). As will be discussed later, both FDA and EMA have released guidances on genetic and pharmacogenetics testing in 2007 and FDA, EMA and PMDA adopted the ICH-E15 guideline, but none of these guidelines specifically address ADME genotyping platforms or quality. Recently the FDA announced the draft guidance on laboratory developed tests that could lead to more specific guidance on the topic [29]. Quality considerations usually have to be adopted from practices such as Good Clinical Laboratory Practices (GCLP), Good Laboratory Practices (GLP) and bioanalytical laboratory guidance. An overview of the different laboratory practices and the associated standards and guidance's are listed in Table 2.

Since the survey in 2006, where 93% of companies utilized internal laboratories to perform their ADME genotyping, there has been a shift towards a greater use of Contract Research Organizations (CROs). Sixty-three percent of the companies queried in the present survey use external laboratories exclusively for their ADME PGx testing, while 37% use a combination of internal and external facilities (Q10). The two reasons given most frequently for using an external laboratory were lack of internal resources and laboratory certification. The majority of companies (86%) using external laboratories indicated that they have used laboratories that apply GCLP practices to sample collection and generation of genotyping data in clinical trials. Seventy-three percent use laboratories that have CLIA accreditation, while half use laboratories that have CAP or ISO accreditation. Sixty-four percent of companies use laboratories that apply GLP practices (Q25). As these percentages add up to more than 100%, companies find more than one of these

Figure 2. The number of major ADME genes genotyped by Industry Pharmacogenomics Working Group member companies.

laboratory accreditation practices appropriate. Sixty-seven percent of companies indicated that they always use a certified laboratory when ADME PGx is required for inclusion/exclusion criteria in a clinical trial (Q26). Other reasons that companies use external laboratories for genotyping included expertise and quality/performance characteristics.

For those companies that have internal genotyping facilities, all indicated that they use Taqman ADME assays (Life Technologies). Three companies use the Affymetrix DMET™ Plus Array and/or Sanger sequencing (Q12). Other platforms used for internal genotyping include pyrosequencing, Illumina's BeadChip Array, Roche's AmpliChip® CYP450 Test and Droplet Digital™ PCR (ddPCR™). Cost and ease of use were the top reasons given by most companies for their choice of platform (Q13). Other reasons included through-put, number of markers and sensitivity. The pros and cons of each platform as viewed by the authors are summarized in Table 3.

In the past decade, NGS (Next Generation Sequencing) platforms have been developed which are inexpensive and robust enough to have found applications in both research and clinical labs. Out of the five companies who indicated NGS or GWAS have impacted the practice of PGx at their company (Q32), one company has implemented NGS technology in PGx; while the other companies are in evaluation or consideration for using NGS. Compared with array-based genotyping platforms, like DMET array or GWAS (Genome Wide

Association Studies), NGS has the advantage of being able to genotype a known functional variant, as well as aid in the discovery of uncharacterized functional variants.

Specific regulatory interactions during drug development

In the 2006 survey, 10 out of 14 PhRMA members indicated that their companies had interacted with regulatory authorities regarding ADME PGx. For the current survey we intended to capture regulatory interactions in more detail and asked whether regulatory authorities requested/suggested sample collections in clinical development programs during review meetings. Such requests would provide additional evidence to the strong recommendations expressed in recently issued guidelines by both the FDA and EMA. Six companies replied with 'Yes' when we asked whether or not regulatory authorities had requested or suggested to them that they collect DNA samples. Eight companies had not received requests to collect samples and 3 companies did not provide an answer (Q27). When asked if regulatory authorities requested/suggested analysis in clinical development programs during review meetings, five companies replied with 'Yes' and nine answered with 'No' (Q28). Nine companies received requests or suggestions for additional analyses on stored samples (Q17). This indicates that regulatory authorities are actively encouraging pharmaceutical companies to implement PGx strategies in their

development program, if these strategies do not already exist.

Key resources

Regulatory guidelines usually recommend testing of ‘known functionally polymorphic enzymes’ or ‘well-established variants’ when a large effect on exposure is expected. While the FDA lists more than 160 examples of PGx biomarkers in approved drug labels on their webpage, it is not always clear what variations may be relevant in an emerging clinical development program. A multitude of curated and uncurated sources are available for use when it comes to selecting alleles and SNP’s for pharmacogenetic analysis.

Our survey did not show preferences for curated data sources such as PharmGKB (73%) and published literature (93%) over uncurated sources such as dbSNP (87%) or the 1000 genome project (80%). The selection of alleles and SNP based on technical platforms was only carried out by a much smaller percentage of respondents (33%), which probably reflects the more restricted application in specific projects (Q29). NGS, ENSEMBL, gene-specific nomenclature pages [30] and

Pharmaaddme [31] were all named as other potential resources by member companies.

While the use of nonannotated data sources, such as dbSNP and the 1000 genome project for allele selection were reported with similar frequency as annotated data sources, the latter were preferred for the interpretation of results. Nearly all companies relied on literature for interpretation and 75% would at least occasionally use PharmGKB or platform specific methods (56%) (Q30). Pharmacogenomics strategies in the early phase of clinical development will often be challenged by the unknown substrate specific impact of available variations and different frequency of variants in the trial and specific indicated populations. This poses challenges in the establishment of a comprehensive variant panel for genotyping which could be applied across all early stage studies. However, efforts in the scientific community such as the ‘Very Important PGx (VIP) gene summary pages’ on the Pharmacogenomics Knowledge Base web site [32] or the ‘Clinical Pharmacogenetics Implementation Consortium’ [9] guidelines and publications, which aggregate published information on many genes, should facili-

Figure 3. Comparison of the percentage of responses indicating the ADME genes that were genotyped by major Pharmaceutical companies in 2006 and 2013.

Table 2. A list of currently applicable regulations and guidance's for clinical pharmacogenomics.		
Laboratory practice		Standard or guidance
GCLP	<ul style="list-style-type: none"> • Supports human sample analysis • Supports result reporting to medical professionals • Results may be for diagnosis and/or treatment of patients or clinical research subjects • Testing methods employed typically are government approved <i>in vitro</i> diagnostic devices 	<ul style="list-style-type: none"> • CLIA • CAP • ISO
GLP	<ul style="list-style-type: none"> • Supports nonhuman laboratory analysis • Samples are primarily derived from animal host systems • Analysis of animal samples, which support nonclinical research studies 	<ul style="list-style-type: none"> • GLP for nonclinical laboratory studies
Bioanalytical laboratory practice	<ul style="list-style-type: none"> • Supports analysis of human research samples • Results are not intended to be reported to physicians for the purpose of diagnosis and/or treatment 	<ul style="list-style-type: none"> • US FDA Guidance for Industry Bioanalytical Method Validation • EMA Guideline on Validation of Bioanalytical Methods
CAP: College of American Pathologists; CLIA: US Clinical Laboratory Improvement Amendments regulation 42 CFR 493; GCLP: Good clinical practice; GLP: US Code of Federal Regulations Title 21, Part 5; ISO: International Standards of Operation 15189.		

tate the implementation of pharmacogenomics strategies in the future.

Discussion: I-PWG perspective

In the ensuing 7 years since the 2006 survey among pharmaceutical companies, more companies routinely collect and store genetic samples from early clinical trials. Although this trend is positive, there remains significant room for improved adoption as standard practice across I-PWG member companies. This is especially important given the potential retrospective testing to address emerging issues during drug development in addition to prospective (hypothesis driven) testing. Although the scope of this perspective is specifically to address genotyping of ADME genes as a determinant of PK variability, the collection and long-term storage of DNA samples for retrospective testing is driven by the larger interests. This includes the examination of pharmacogenomics relating to drug efficacy and safety as well as potentially the use of biobank samples for more general research purposes.

After more than a decade of symposia and publications on the impact of pharmacogenomics in pharmacokinetics, efficacy and safety, with some emphasis on ADME PGx due to the greater maturity of the science, a more differentiated view about the value of PGx information impacting PK variability is reflected in the declining number of companies stating that such information has been used in internal decision making during clinical stages of drug development (Q19). The greatest frequency of testing occurs in Phase 1, including drug–drug interaction studies, studies which have rich PK data (full concentration-time course profiles). In the conduct of DDI studies, because interactions

may differ in subgroups of different genotypes, regulatory guidance recommend consideration of genotyping of subjects [33]. ADME genotyping is infrequently conducted in Phase 2 studies, where typically an association of genotype or phenotype and clinical endpoints of efficacy and safety would be studied, and only one in four companies had ever employed ADME genotyping for dose adjustment in a clinical trial. The collection of DNA from Phase 2 and Phase 3 studies for retrospective analysis of genotypes of interest is something that could provide valuable insights into the function of newly described clinically significant variants. The broad consent required for the long-term storage of these samples, however, could be a source of constraint for the acquisition and storage of these sample sets.

Significance of ADME PGx as a factor in PK variability

The EMA guideline for prospective genotyping during Phase1 trials if the contribution of a polymorphic enzyme constitutes >50% of total CL (drug clearance) is certainly a useful guidance approach in early studies, as exposure would be expected to increase approximately twofold for complete loss of function variants. The EMA further states that ADME genotyping must be undertaken if >25% of the total clearance is via a polymorphic enzyme based on *in vivo* data. The majority of the companies agree with EMA guidance thresholds for including ADME genotyping in clinical trials, and would also conduct retrospective genotyping when high PK variability or observation of a PK outlier, consistent with the guidance. However, in practice, this is not reflected in either planning or widespread testing during Phase 1.

There are a number of potential reasons for limited ADME testing in early phase clinical development:

- It appears that the majority of development candidates are not thought to meet that criterion,

Table 3. Comparison of example assays used for clinical pharmacogenomics.		
Platform [†]	Pros	Cons
Allele-specific assays		
TaqMan® DME Assays	<ul style="list-style-type: none"> • Single marker assay • Flexible • Validated by Vendor for workability • Coverage of majority of the key clinical ADME markers 	<ul style="list-style-type: none"> • Limitations of the technology do not allow for analysis of key variants including CNV • Broad differences among different assays being used by different laboratories
Panel-based approaches		
Affymetrix DMETPlus Array	<ul style="list-style-type: none"> • Broad coverage of ADME polymorphisms • 1936 markers in 225 genes 	<ul style="list-style-type: none"> • Cannot efficiently detect CNVs • Potential homozygous mutants not called by software
Roche AmpliChip	<ul style="list-style-type: none"> • 510(K) cleared • Validated for CYP2D6 	<ul style="list-style-type: none"> • Limited to CYP2D6 and CYP2C19 only • Does not include the CYP2C19*17 allele • Limitations to the genotype calls based on the software's interpretation table
VeraCode ADME Core Panel (microbead)	<ul style="list-style-type: none"> • 184 biomarkers in 34 genes • Medium coverage of ADME polymorphisms 	<ul style="list-style-type: none"> • Cannot efficiently detect CNVs
iPLEX ADME PGx panel (Sequenom)	<ul style="list-style-type: none"> • 192 biomarkers in 36 genes • Medium coverage of ADME polymorphisms • Mass-spectrometry based and high sensitivity for rare somatic mutations 	<ul style="list-style-type: none"> • Limited flexibility for incorporation of new assays • Technical work stream is challenging for use in regulated environments
Sequencing-based approaches		
Sanger sequencing	<ul style="list-style-type: none"> • US FDA gold standard for sequencing • Capable of genotyping most of ADME variants and discovering novel variants 	<ul style="list-style-type: none"> • Cannot adequately determine large In/Del's or CNVs
Next-generation sequencing like RainDance ADMESeq™, and Targeted seq or Commercialized Exome Sequencing via Illumina, Ion Torrent or SOLiD [‡]	<ul style="list-style-type: none"> • Coverage of all ADME genes, including SNVs, In/Del's, and CNVs using one platform • Can detect novel variants across ADME genes of interest 	<ul style="list-style-type: none"> • Bioinformatics and data interpretation challenges, for example, call for *allele or translate to genetically predicted enzymatic activity • The current calling algorithms for larger In/Dels, homopolymer repeats and CNVs need to be improved although those structural variations are able to be called theoretically
[†] The platforms listed in the table were commercially available, and identified by the I-PWG members, some of which are commonly used. [‡] The next-generation sequencing platforms listed in the table were based on a research term of 'next-generation sequencing for genotyping ADME genes' on 10 February 2015. ADME: Absorption, distribution, metabolism, excretion; CNV: Copy number variation; In/Del: Insertion/Deletion; SNV: Single nucleotide variant.		

since 94% of the companies indicated genotyping would be conducted when preclinical data indicated the role of a specific enzyme (with relevant functional polymorphic variation) in the compound's PK. With regard to polymorphism amongst CYP enzymes (e.g., CYP2C9, CYP2C19 and CYP2D6), robust *in vitro* assays employed during drug discovery and candidate selection allow teams to make this a selection feature, such that fewer compounds are going into development with high dependence for a single pathway that has functional polymorphic variation;

- The vast majority of member companies take a hypothesis-testing approach in prospective genotyping and many small molecule candidates have CYP3A as the major metabolic enzyme family [22]. While CYP3A5 has a well-known loss-of-function allele (*3), few drugs exhibit a bimodal distribution or exposures of greater than twofold change based on CYP3A5*3 genotype. The reason for this may be due to the overlapping substrate specificity with CYP3A4 and the intrinsic wide variability of 3A activity related to genetics and environmental exposure [34]. Nor has there been compelling evidence for a functional polymorphism in CYP3A4;
- A standard approach to clinical confirmation of the major clearance pathway based on *in vitro* evidence has been a drug–drug interaction study in a limited number of subjects, rather than genotyping, due to the availability of selective and potent inhibitors of CYPs;
- In a cost constrained, high attrition stage of clinical development, identifying the major clearance mechanism may be positioned later in development, as resources are prioritized to quickly take compounds to proof of concept studies with increasing investments in mechanism or efficacy-based biomarkers.

Quality considerations for ADME genotyping & emerging trends

A previous industry survey in 2006 showed that a vast majority of member companies maintained internal laboratories for PGx genotyping studies. That is in marked contrast to the nearly half of respondents in the 2014 survey who rely solely on the use of external laboratories for genotyping studies. This development has been partly driven by the availability of robust and low cost genotyping services by external service providers, similar to other aspects of development operations (e.g., clinical conduct and drug bioanalysis) [35].

Currently, the FDA and the EMA have quality guidance which are applicable to genotyping and data anal-

yses in support of PGx in clinical trials. In 2007 the FDA issued 'Pharmacogenetic Tests and Genetic Tests for Heritable Markers' guidance, addressing the critical parameters of tests that are in principle applicable to other diagnostic platforms (e.g., specificity, sensitivity, accuracy) but are clearly referring to platforms such as sequencing, PCR and arrays. The EMA published similar guidance in 2007: 'Reflection Paper on Pharmacogenomic Samples, Testing and Data Handling'. In 2010, the FDA, EMA and PMDA collectively adopted the 'E16 Biomarkers Related to Drug or Biotechnology Product Development: Context, Structure, and Format of Qualification Submissions', which gives further guidance on the qualification process. These documents do not dictate specific acceptance criteria, but merely deal with a work flow description to technically validate a platform and qualify the markers in a clinical context. Therefore, it is not surprising that laboratory certification is the other main reason given by member companies for the use of external service providers. Over two-thirds of member companies use laboratories which have CLIA accreditation or a combination of other quality certifications (CAP, ISO or GLP).

Recently (2014), the FDA released a draft 'Framework for Regulatory Oversight of Laboratory Developed Tests' for public comment, with the objective to provide doctors and patients access to safe, accurate and reliable diagnostic tests. This draft guidance would bring laboratory developed tests (LDTs), which have traditionally been overseen by CLIA, under FDA enforcement and premarket review. As the majority of pharmacogenomic testing is currently done under the LDT provision in CLIA, the increased oversight by the FDA will have an impact on this testing (although the extent of this impact will depend on the final FDA guidance). The draft guidance proposed a risk-based matrix approach to regulating LDTs, where high risk LDTs (Class III) will have enhanced oversight by the FDA and be required to register as a medical device with premarket review. Moderate (Class II) and low (Class I) risk LDTs will be required to register with the FDA (although direct oversight of these is currently unclear, and may involve third parties external to the FDA itself for regulation). It remains to be seen how the current practice of genotyping for clinical studies in early development will be influenced in this changing regulatory environment.

Current NGS technologies allow for the generation of high quality sequencing across reasonably long read lengths (~150 bps). As NGS technologies advances and pricing continue to drop, NGS has emerged as a powerful tool with significant potential for effectively and comprehensively investigating common and rare genetic variants in ADME genes. For example, NGS can be used for a PGx case study of PK outlier or observations of unex-

plained PK differences between race/ethnicities as drugs develop globally. NGS has demonstrated reasonable reliability in terms of genotyping genetic variants for ADME genes, especially single nucleotide variants (SNVs) and small insertion/deletions (InDels) that would be missed with current TaqMan genotyping approaches. Larger InDels and gene copy number variations (CNVs) can also theoretically be identified, although the current calling algorithms still need to be improved to increase confidence in calling those structural variations [36]. In the next 5 years NGS could either replace current technology platforms or exist in parallel as a screening tool to be utilized with outliers which have no known variant detected by more allele-specific assays [37].

Pharmacogenomics & drug development

Genotyping to guide dosing or actual drug selection (actionable steps) occurs only for a minority of drugs (Table 1). This may be due to the fact that other factors influence exposure differences amongst patients, such as the actual fraction of total clearance represented by the polymorphic enzyme or transporter and the degree of functional changes resulting from the genetic change. The importance of genotype in assessing exposure (and ultimately efficacy and safety) in clinical trials is similar to inclusion of concomitant drugs which are strong inhibitors of the polymorphic enzyme, which would create a poor metabolizer phenotype. The interrelationship of DDI and genetic polymorphisms in drug enzymes and transporters is increasingly reflected in a concordance in information contained in a drug product label [14]. By the strictest definition of drug–gene associations with actionable steps (drug selection or adjusting the dose based on phenotype) established by the CPIC, only nine guidelines had been published as of their 2014 review article [21], with only six relating to ADME genes. In comparison, a similar undertaking to provide actionable guidance for drug–gene pairs has been undertaken by the Royal Dutch Association for the Advancement of Pharmacy, whose working group by 2011 had compiled therapeutic (dose) recommendations for 163 genotype/phenotype–drug combinations reflecting 53 drugs and 11 genes [38]. For other drugs without actionable steps, information in the Product Label demonstrates a consideration of ADME PGx was made during clinical development. The current number of drugs with actionable guidance by the CPIC or Royal Dutch Association for the Advancement of Pharmacy – Pharmacogenetics Working Group is indicated in Table 1.

Conclusion

We view the evaluation of ADME polymorphism on drug exposure in early clinical stages as a contributing step in the overall definition of major intrinsic and extrinsic fac-

tors influencing drug exposure. Important information is typically obtained by the conduct of a specific drug–drug interaction study with a strong inhibitor for the proposed major clearance pathway, based on available *in vitro* data, and if available, human *in vivo* information from Phase 1 studies. This includes the percent excreted in urine unchanged, major circulating and urinary metabolites from Phase 1 and a definitive radiotracer ADME/mass balance study defining metabolic pathways and prediction of CL mechanisms (enzymes) based on chemical structure and further *in vitro* studies [39]. These factors will define the impact of a polymorphic pathway in perspective of the overall degree of PK variability. These studies are typically complete by the end of Phase 2, so as to have available for discussion with Regulatory agencies regarding Phase III trial designs.

Future perspective

With the reduction of cost in obtaining patient genetic data as well as further understanding of clearance pathways and gene function in clinical trials, we are expecting increased ADME genomic information in clinical trials. Furthermore, with the rapid development of technologies in nucleotide analysis, we are expecting deep and broad understanding of genetic contribution to pharmacokinetics and pharmacodynamics in drug development. However, there will be a need to correlate genotype and phenotype, as additional mutations identified through full genome sequencing (including promoter and intronic regions). As a component of regulatory focus on laboratory developed tests, we expect more definitive guidance on the assay validation and verification steps of ADME genomic testing. Due to the multiple intrinsic and extrinsic factors that contribute to inter-subject and intrasubject variability, only for a minority of drugs will single ADME gene phenotype guide drug selection or dose adjustment. More commonly, such information can contribute to algorithms for dose selection of narrow therapeutic index drugs.

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Executive summary

Pharmacogenomics impacting pharmacokinetics variability

- Understanding the intrinsic and extrinsic factors underlying variability in exposure between individuals is a key objective during the early stages of clinical development.
- Genetic polymorphisms in metabolizing enzymes and drug transporters have been shown to significantly impact the exposure of drugs having a high dependence on a single mechanism for their absorption, distribution, clearance (metabolism, renal or biliary excretion) or kinetics (formation or clearance) of an active metabolite (ADME).
- The Industry Pharmacogenomics Working Group conducted a survey of members to assess the impact of the recent Regulatory Guidance issued by the US FDA and EMA on industry practices.

Role of regulators in drug development

- Regulators are influencing the incorporation of genotyping in early clinical trials through public hearings, publications and issuance of guidance.

DNA sample collection & storage

- The majority of companies always or usually collect DNA for ADME-related studies in Phase 1 studies (Q1), but less frequently in patient trials (Phase 2).

Pharmacogenomic testing during early clinical trials

- Two-thirds of the companies agree with the EMA guidance thresholds to incorporate pharmacogenomics testing.
- The majority of companies include genotyping in studies based on data-driven decisions including pre-clinical information, and this results in a low frequency of testing during early stages of clinical development.

Clinical study design

- ADME genotyping was most commonly used for inclusion or exclusion decisions in Phase 1 studies, and in particular for DDI and escalating multiple dose studies.

ADME pharmacogenomic assay considerations

- Gene variants for *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *UGT1A1* and *SLCO1B1* are most commonly tested.

Assay platform & quality considerations for ADME genotyping

- Currently, there is no regulatory guidance on the platforms or quality considerations for ADME genotyping in clinical trials, although this may change with the recently announced FDA draft guidance on laboratory developed tests.
- Quality considerations usually have to be adopted from practices such as Good Clinical Laboratory Practices, Good Laboratory Practices and bioanalytical laboratory guidance.

Specific regulatory interactions during drug development

- Regulatory authorities are actively encouraging pharmaceutical companies to implement pharmacogenomics strategies in their development program by specifically requesting sample collection and additional analyses in clinical development programs.

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