Review

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Pharmacogenomics

Assessment of drug metabolism enzyme and transporter pharmacogenetics in drug discovery and early development: perspectives of the I-PWG

Genetic variants of drug metabolism enzymes and transporters can result in high pharmacokinetic and pharmacodynamic variability, unwanted characteristics of efficacious and safe drugs. Ideally, the contributions of these enzymes and transporters to drug disposition can be predicted from *in vitro* experiments and *in silico* modeling in discovery or early development, and then be utilized during clinical development. Recently, regulatory agencies have provided guidance on the preclinical investigation of pharmacogenetics, for application to clinical drug development. This white paper summarizes the results of an industry survey conducted by the Industry Pharmacogenomics Working Group on current practice and challenges with using *in vitro* systems and *in silico* models to understand pharmacogenetic causes of variability in drug disposition.

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Survey of current industry practices & challenges

Recently, regulatory agencies in the USA, EU and Japan have provided greater guidance for assessment of pharmacogenetics (PGx) during drug development, as formal Guidances and also Perspectives published in scientific journals [1]. The Industry Pharmacogenomics Working Group (I-PWG; [2]) is a working group, which is open to any company within the Pharmaceutical Industry and is presently comprised of 21 member companies. A Task Force conducted a survey among member companies to assess practices relating to absorption, distribution, metabolism and excretion (ADME) PGx in preclinical drug development. Objectives of the survey were to: first, understand the use of *in vitro* systems and *in silico* modeling in the pharmaceutical industry for evaluating the contribution of drug metabolism enzymes and transporters in new compound disposition; second, assess use of *in vitro* data and *in silico* models to quantitatively estimate pharmacokinetic (PK) variability of new chemical entities due to genetic variation in ADME-related genes, and then to incorporate that into clinical development plans; third, provide an I-PWG perspective on challenges with current practices and fourth, use the results to engage in further discussion between pharmaceutical scientists, academics and regulatory agencies on scientific opportunities, as well as future updates on PGx guidances.

The survey was completed in the fall of 2013 by 17 member companies. The results provide a representative snapshot of current practice in the industry. As a measure of size, Research and Development budgets of three companies were less than US$ 1 billion, two were between US$ 1 and 2 billion and 12 were more than US$ 2 billion. The complete results of the survey are available in Supplementary Material 1. This article does not necessarily reflect the views of the companies that are members of the I-PWG.

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Fractional drug disposition via drug metabolism enzymes or transporters

The key determinant to predict the quantitative impact of genetic variants in drug metabolism enzymes or transporters on variability of PK or pharmacodynamics (PD) involves accurate estimation of the fractional clearance from individual enzymes (f_m) or transporters (f_t) contributing to the overall clearance of a drug. Conceptually, this can be viewed as analogous to the drug being subjected to a drug interaction with an inhibitor, which mimics the loss or decrease in function associated with the variant enzyme or transporter involved in disposition of the drug [3]. Figure 1 shows the dependence of changes in exposure (area under the curve (AUC) ratio) on f_m or f_t when genetic variation results in partial or complete loss of activity. For example, the systemic exposure of atomoxetine in CYP2D6 poor metabolizers (PMs) was approximately tenfold higher than in CYP2D6 extensive metabolizers (EMs), while coadministration of atomoxetine with strong CYP2D6 inhibitors such as fluoxetine, paroxetine and quinidine to CYP2D6 EM increased the plasma AUC of atomoxetine by six- to eightfold [4], consistent with the *in vitro* CYP2D6 f_m estimate of 96.7% in human liver microsomes (HLM) [5].

Phenotyping drug metabolism enzymes & transporters *in vitro*

Experiments using human *in vitro* systems to investigate the involvement of enzymes and transporters in the disposition of a compound are often called 'reaction phenotyping.' Investigations usually start early in discovery and grow more sophisticated and quantitative as compounds progress as drug candidates, so that f_m or f_t for individual enzymes or transporters can be estimated. Experiments in preclinical species may also be conducted to estimate the relative contribution of clearance (CL) by the gut, liver or kidney of parent drug and metabolites. Incorporating these data into *in silico* models allows assessment of the risk whether a compound could be affected by administration of concomitant drugs (drug–drug interaction [DDI]) or genetic variation, such as SNPs. Supplementary Table 1 summarizes literature examples of the correlation between *in vitro* and clinical data for polymorphic transporters and enzymes. The EMA Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products considers a metabolic pathway important if *in vitro* data suggest that more than 50% of a drug is cleared via a single polymorphic enzyme ($f_m \geq 0.5$) [8], consistent with the EMA Drug Interaction Guideline [9]. The US FDA DDI Guidance recommends evaluating the impact of a polymorphic enzyme or transporter if it is involved in 25% or more of a drug's clearance [10]. The agencies do not specify cutoff values for transporter substrates [1]. An important assumption in specifying cutoff values with preclinical data is that f_m and/or f_t can be estimated reliably in the absence of clinical data on the pharmacokinetics of parent drug and metabolites.

In vitro phenotyping studies (Q5) were conducted by 15 of 17 companies to test for polymorphic enzyme or transporter involvement in clearance of compounds prior to Phase 1 clinical studies. All responding companies (Q7) phenotype for CYP2C9, -2C19 and -2D6, and more than 50% also assess other CYP enzymes (CYPs), various uridine diphosphate glucuronyltransferases (UGTs) and transporters such as P-glycoprotein (P-gp; MDR1; ABCB1), the breast cancer resistance protein (BCRP; ABCG2) and the organic anion transporting polypeptides (OATPs; solute carrier organic anion [SLCO])-1B1 and -1B3. This reflects current understanding of the significance of enzyme and transporter polymorphisms. The *in vitro* systems used most frequently for reaction phenotyping of CYP and UGT (Q8) are HLM (100 and 50%, respectively) and recombinant enzymes (94 and 81%, respectively). For transporters, transfected cell lines (81%), established cell lines such as Caco-2 (75%), membrane vesicles (69%) and human hepatocytes (44%) are used. In addition, seven companies have conducted experiments with *in vitro* systems expressing exomic variants of enzymes or transporters. This was done with CYPs using recombinant enzymes or in HLM from genotyped donors (Q10). UGTs, sulfotransferases or SLCO1B1 variants were most often tested using recombinant systems. Of course, this approach is not useful to investigate synonymous SNPs or variants in intronic gene regions. Widespread use is not expected since most of these reagents are not commercially available.

An equal number of companies (Q7) use data from late discovery assays (e.g., with standardized protocols and limited controls) or from more rigorous 'GLP-like' assays (e.g., high number of calibration samples and replicates, positive and negative controls with characterized compounds) when determining whether to genotype subjects in Phase I clinical trials (Q11). Only one responder used GLP and no one reported using high-throughput assays. Formal reports of *in vitro* experiments, sufficient for regulatory submission, were written by nine of 15 responders (Q12).

Based on the survey (Q6), 60% of companies consider themselves to be acting generally consistent with the regulatory guidances with the others moving in that direction. However, responders to the survey noted several issues that complicate using *in vitro* data to quantitatively predict disposition of drug candidates

(Q26). These included limited availability and functionality of some *in vitro* systems, lack of data needed for mathematical models and incomplete mathematical models. In the following sections, we summarize the current methods utilized for reaction phenotyping of enzymes and transporters.

CYP enzymes

As recently reviewed, a combination of genetic and nongenetic host factors, and environmental factors result in a range of activity of up to 100-fold within a population in the activity of CYPs [11,12]. The clinical consequences of prevalent genetic variants of CYP enzymes are relatively well understood. Based on survey responses (Q27), companies considered the following polymorphic CYPs 'very relevant' (percentage of companies, with 15 responding in parenthesis): CYP2C8 (6.7%), CYP2C9 (47%), CYP2C19 (60%), CYP2D6 (86%), CYP3A4 (20%) and CYP3A5 (6.7%). As excellent reviews and websites are available on the relevance of various genetic variants in *CYP* genes, they are not discussed here [11–15].

CYP phenotyping evaluation can be conducted in various ways [16–19]: first, inhibition with CYP isoform-selective chemical inhibitors and/or inhibitory antibodies in HLM preparations pooled from many individuals (permitting an estimate of 'population average' within a single HLM sample); second, inhibition with chemical inhibitors in human fresh or cryopreserved hepatocytes and third, use of individual recombinant human CYPs. Reagents for some, but not all CYPs (especially variants), are commercially available from a range of vendors.

The contribution of individual CYPs involved in the metabolism of a test compound can be estimated with recombinant CYP systems using a relative activity factor (RAF; Equation 1, Table 1). This is determined by measuring the disappearance of the compound or by following the formation of metabolites [18,20]. Disappearance of compound is typically utilized early in discovery and can be used to identify major drug metabolism pathways. Following the formation of metabolites requires either radiolabeled drug or specific assays and is typically used later in discovery for compounds that are slowly metabolized or to follow an individual metabolism pathway. Assumptions for the RAF approach are that enzyme K_m values and free drug concentrations are independent of the test system employed. Caveats of using recombinant enzymes are that activity can be affected by the expression system (e.g., baculovirus-infected insect cells, yeast or *Escherichia coli*), buffer type (e.g., phosphate or Tris) and the relative expression levels of P450 oxidoreductase and cytochrome b5. Another approach to account for the different intrinsic activity per unit amount of CYP between recombinant enzymes and the liver is to use intersystem extrapolating factors (ISEFs; Equation 2, Table 1; [21]). ISEF accounts for the difference in activity per unit enzyme from those due to CYP abundance, allowing investigation of population variability in metabolic clearance. From Equation 2 (Table 1), intrinsic clearance (Cl_{int}) is estimated using values for each recombinant CYP isoform (Equation 3). Since clearance values are additive, the sum of Cl_{int} determined for each CYP provides the total CYP Cl_{int} (Equation 3). By dividing Cl_{int} for an individual isoform by the total Cl_{int}, f_m for that isoform is estimated (Equation 6, Table 1). For example, the general applicability of this approach for phenotyping studies was described [22], showing that for ten marketed compounds the Cl_{int} values obtained using the ISEF method correlated well with the measured Cl_{int} in HLM. However, this approach is not always successful, such as for investigational compound LY2878735, when the f_m prediction for CYP2D6 was less than 30% but in the clinic was approximately 70% [23]. Cases like this show the difficulties with making predictions based on *in vitro* data only. The RAF and ISEF approaches can be utilized for other enzymes and transporters, if necessary information, and selective probe substrates and reagents are available.

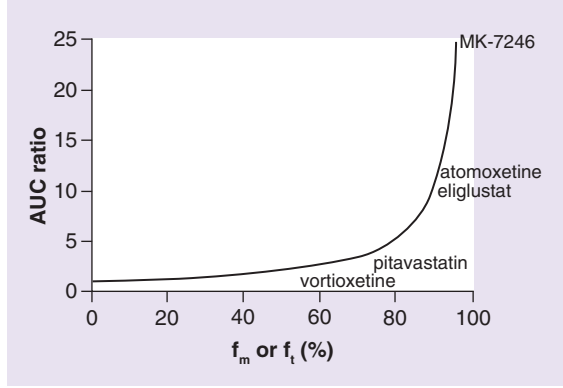

Figure 1. Change in AUC ratio as a function of f_m or f_t, the fraction of the disposition by the enzyme or transporter pathway involved. For atomoxetine, eliglustat and vortioxetine, the AUC ratio is of CYP2D6 poor metabolizers, compared with extensive metabolizers [PRESCRIPTION INFO]. For pitavastatin, the AUC ratio is of *OATP1B1*15* homozygotes, compared with wild-type *OATP1B1* [6]. For MK-7246, the AUC ratio is of *UGT2B17*2* homozygotes, compared with wild-type *UGT2B17* [7]. The observed AUC ratios were estimated by fitting to the f_m as $1/(1–f_m)$, assuming disposition by only the pathway effected.
AUC: Area under the curve; f_m: Fractional clearance from an indiviual enzyme; f_t: Fractional clearance from an individual transporter.

UDP-glucuronosyltransferases

UGT-mediated glucuronidation is a significant metabolic pathway for many drugs with large inter-

Table 1. Equations commonly used in phenotyping studies for drug metabolizing enzymes and transporters.

Equation	Application	No. in text
$RAF\,(V_{max}) = \frac{V_{max}\,(HLM)}{V_{max}\,(rhCYP)}$	Scale contribution of individual CYP to metabolism from recombinant enzymes to HLM. For liver transporter studies, HLM and rhCYP would be replaced by hepatocytes and recombinant cell lines, respectively	1
$Cl_{int}\,ISEF = \frac{CL_{int}\,(HLM)}{CL_{int}\,(rCYP) \times CYP\ abundance\,(HLM)}$	Dimensionless scalar to convert contribution of enzymes from individual recombinant enzymes to HLM	2
$CL_{int} = \left(\sum_{j}^{n} ISEF_j \times CL_{int\,rCYPj} \times [P]_j\right) \times MPPGL \times LW$	Scale contribution of all enzymes contributing to metabolic clearance	3
$CL_{hepatocytes} = CL_{OATP1B1,\,in\,vitro} \cdot RAF_{OATP1B1} + CL_{OATP1B3,\,in\,vitro} \cdot RAF_{OATP1B3}$	Determine CL of OATP-1B1, -1B3 substrates using the relative activity approach	4
$CL_{hepatocytes} = CL_{OATP1B1,\,in\,vitro} \cdot REF_{OATP1B1} + CL_{OATP1B3,\,in\,vitro} \cdot REF_{OATP1B3}$	Determine CL of OATP-1B1, -1B3 substrates using the relative expression approach	5
$f_m = \frac{CL_{int\,(enzyme\ j)}}{\sum_{j}^{n} CL_{int}}$	Fractional clearance through an individual enzyme (or transporter)	6

Cl_{int}: Intrinsic clearance; $Cl_{int\,rCYPj}$: pmol of product/min/pmol for CYPj; CL: Clearance; HLM: Human liver microsome; ISEF: Intersystem extrapolation factor; j: CYP isoform tested; LW: Liver weight; MPPGL: Microsomal protein per gram of liver; [P]j: pmol CYPj/mg microsomal protein; RAF: Relative activity factor; REF: Relative expression factor; rh: Recombinant human; V_{max}: Maximum rate of metabolism or transport of a probe substrate by an individual enzyme or transporter.

individual variation in clearance due to UGT genetic polymorphism. UGT involvement is becoming increasingly important in drug development as CYP-mediated metabolism is reduced by chemical manipulation in discovery [19]. This is exemplified by recent examples in which a UGT2B17 deletion or a UGT2B10 splicing variant resulted in PK variability of investigational compounds [7,24]. Regulatory agencies have also recognized the importance of UGT enzymes in drug development, including them in the EMA and draft FDA DDI Guidances. The survey showed that UGT enzymes have been examined at the preclinical stage by eight of 17 respondents (Q7). UGT1A1 was considered very relevant (67% from Q27) and the most studied, compared with other UGTs, whereas the assessment for relevance of UGT-1A4, -1A6, -1A9, -2B7, -2B15 and -2B17 varied across the companies.

Current *in vitro* systems for estimating UGT f_m are limited [25–28]. Recommendations have been published to establish standardized and robust *in vitro* UGT assay conditions, which are more complex than for CYP [19,29–30]. Isoform-selective substrates and inhibitors for *in vitro* use have not been identified for many UGT isoforms (Table 2). Recently, an example was published applying the RAF approach to successfully predict the drug clearance of the UGT substrate laropiprant [31]. Six of 17 respondents indicated that RAF or ISEF approaches had been utilized, although use of ISEF with UGTs has not been published (Q14).

Because of limitations or unavailability of *in vitro* systems, predicting the effects of *UGT* gene variants on PK and PD of new compounds is difficult. Based on two reviews describing the effect of *UGT* genetic variants on activities [33,41] and a detailed description of *UGT* polymorphisms [57], the I-PWG task force categorized functionally relevant *UGT* variants (Table 3). This reference can be used for prioritizing *UGT* genotypes to investigate clinically, and delineating genotype–phenotype relationships of drugs under investigation.

Carboxylesterases

Carboxylesterases (CES) metabolize several prodrugs to active therapeutic agents or convert drugs to inactive metabolites [58]. Based on amino acid homology, human CES are classified into five families, hCES1–hCES5, with the two major CES identified as hCES1 and hCES2 [58]. The liver mainly expresses hCES1 with lower amounts of hCES2, whereas the small intestine expresses hCES2, but not hCES1. Large interindividual variability in the clearance of CES substrates has been observed *in vitro* [59,60]. The mechanisms underlying this variability are not understood, although genetic variation is presumed to contribute.

Table 2. Selective probe substrates and inhibitors commonly used in the pharmaceutical industry for *in vitro* CYP, UGT, and drug transporter phenotyping studies.

Enzyme or transporter	Probe substrates for *in vitro* studies (marker reaction)	Inhibitors for *in vitro* studies	Ref.
CYP enzymes[†]			[19]
CYP1A2	Phenacetin (*O*-deethylation)	Furafylline	
CYP2B6	Bupropion (hydroxylation)	2-Phenyl-2-(1-piperdinyl)propane (PPP)	
CYP2C8	Taxol (6-hydroxylation), amodiaquine (*N*-deethylation)	Montelukast	
CYP2C9	Diclofenac (4′-hydroxylation)	Sulfaphenazole	
CYP2C19	*S*-Mephenytoin (4′-hydroxylation)	Benzylnirvanol (-)-*N*-3–benzyl-phenobarbital	
CYP2D6	Dextromethorphan (*O*-demethylation) Clomiphene for multiple SNPs	Quinidine	
CYP3A4/3A5	Midazolam (1′-hydroxylation), testosterone (6β-hydroxylation)	Ketoconazole, azamulin	
CYP3A4	Midazolam (1′-hydroxylation), testosterone (6β-hydroxylation)	1-Methyl-3-[1-methyl-5-(4-methylphenyl)-1*H*-pyrazol-4-yl]-4-[(3*S*)-3-piperidin-1-ylpyrrolidin-1-yl]-1*H*-pyrazolo[3,4-d]pyrimidine (CYP3cide), 1-(4-imidazopyridinyl-7phenyl)-3-(4′-cyanobiphenyl)urea (SR-9186)	
UGT enzymes			[19,30]
UGT1A1	β-Estradiol (*O*-glucuronidation), SN-38 (*O*-glucuronidation)	Nicardipine, erlotinib, atazanavir	
UGT1A3	Zolasartan (*N*-glucuronidation), Hexafluoro-1α, 25-dihydroxyvitamin D_3 (*O*-glucuronidation)	Buprenorphine	
UGT1A4	Imipramine, Haloperidol (*N*-glucuronidation), Trifluoperazine (*N*-glucuronidation)	Hecogenin	
UGT1A9	Propofol (*O*-glucuronidation)	Niflumic acid	
UGT2B7	3′-Azido-3′-deoxythymidine (AZT) (*O*-glucuronidation), Zidovudine (*O*-glucuronidation)	Fluconazole, diclofenac	
UGT2B15	(*S*)-Oxazepam (*O*-glucuronidation)	Ibuprofen	
CES enzymes			
CES1	Clopidogrel (carboxylation)	Bis(4-nitrophenyl phosphate), arachidonic acid	[49,50]
CES2	Irinotecan (formation of SN38)	Loperamide	[51,52]

[†]Substrates and inhibitors also recommended by the FDA [56].
[‡]Also a substrate for OATP-1B1 and -1B3. Selective inhibitors need to be applied to discriminate between these transporters.
Of note, many drugs are only weak or no substrates for OATP2B1.

Table 2. Selective probe substrates and inhibitors commonly used in the pharmaceutical industry for *in vitro* CYP, UGT, and drug transporter phenotyping studies (cont.).

Enzyme or transporter	Probe substrates for *in vitro* studies (marker reaction)	Inhibitors for *in vitro* studies	Ref.
Transporters			[53-55]
OATP1B1	Estrone 3-sulfate, pravastatin, pitavastatin, valsartan	Estropipate, rifampicin, rifamycin SV, ritonavir, lopinavir, bromosulfophthalein, MK-571	
OATP1B3	Cholecystokinin octapeptide, telmisartan	Ursolic acid, and same inhibitors as for OATP1B1	
OATP2B1	Estradiol-17β-D-glucuronide‡	Bromosulfophthalein, MK-571	
NTCP	Taurocholate	Conduct studies in the absence of Na^+, taurocholate	
OCT1	Tetraethyl ammonium 4′,6-diamidino-2-phenylindol	Verapamil, quinidine, Decynium-22, Progesterone	
OCT2	4-(4-(dimethylamino)steryl)-*N*-methyl-pyridinium Metformin Tetraethyl ammonium	Disopyramide Imipramine Tacrine	

†Substrates and inhibitors also recommended by the FDA [56].
‡Also a substrate for OATP-1B1 and -1B3. Selective inhibitors need to be applied to discriminate between these transporters.
Of note, many drugs are only weak or no substrates for OATP2B1.

Heterologously expressed hCES, as well as human liver and intestine fractions have been used to investigate hCES-mediated hydrolytic activity. Scaling of CES activity data from either *in vitro* systems or preclinical species to predict hCES activity has not been well defined and currently is an active research area [61–64].

Genetic variants of hCES1 may affect disposition and clinical effects of CES-substrate drugs as illustrated in Table 4 and in a recent review [65]. Common hCES2 genetic variants (minor allele frequency >5%) have not been observed [66,67].

Other enzymes

Reaction phenotyping studies for aryl amine *N*-acetyl transferases (NAT), aldehyde oxidase and flavin-containing monooxygenases 1 and 3 are conducted on an issue-driven basis across companies (Q7). They are not discussed here in detail as reviews have been published elsewhere [68–71].

Drug transporters

The current FDA and the EMA DDI Guidances recommend that drugs are investigated as substrates and inhibitors of several drug transporters. These include P-gp (MDR1, ABCB1); BCRP (ABCG2); OATPs, (SLCO)-1B1 and -1B3; organic anion transporters (OAT, SLC22)-1 and -3; organic cation transporters (OCT, SLC22)-1 and -2; multidrug and toxin extrusion proteins (MATE, SLC47)-1 and -2K; and the bile salt export pump (BSEP; ABCB11). Except for OCT1, these are aligned with the International Transporter Consortium [72–74]. Survey results from 16 responders (Q7) indicate that most companies investigated P-gp (88%) prior to Phase 1 clinical studies. BCRP and OATP1B1 are also tested frequently (63 and 56%, respectively), but other transporters are analyzed more on a case-by-case basis.

Although genetic variants have been identified in several drug transporters, their functional (and clinical) relevance is not clear in many cases [75,76]. In general, when observed, the effects of transporter SNPs on blood or plasma drug exposure are relatively small (<threefold), although they could be large locally in tissues such as liver or kidney [76]. Most companies considered polymorphisms in OATP1B3, OCT-1 and -2, OAT-1 and -3, BSEP, and MATE1 and MATE2k as only somewhat relevant or 'I don't know', but 93, 53 and 47% of survey respondents considered polymorphisms in OATP1B1, P-gp and BCRP, respectively, as relevant (Q27). Clinical evidence indicates that OATP1B1 (c.521T>C) and BCRP (c.421C>A) are the most relevant known variants [77]. For OATP1B1, the 521T>C SNP resulted in increased exposures of some statins and methotrexate with alterations in efficacy

Table 3. Categorization of clinical significance of SNPs in uridine diphosphate glucuronyltransferase enzymes.

Category	Criteria	UGT	Examples of drugs (UGT involved)	Considerations
1	Strong effects on drug CL (>fivefold change) Or Consistent >1.5-fold change in CL across multiple substrates	*UGT1A1*28* *UGT2B15*2* (D85Y) *UGT2B17*2*	Irinotecan (UGT1A1)† Oxazepam, Lorazepam, Sipoglitazar (UGT2B15)¶ MK-7246 (UGT2B17)#	Investigate the contribution of these UGT variants on the PK of a drug candidate Evaluate the magnitude of DDIs mediated by these UGT enzymes§
2	Inconsistent effects found on CL (>1.5-fold change) Or Weak but consistent effects on CL (<1.5-fold change)	UGT1A3, UGT1A4, UGT1A6 and UGT2B7	Atorvastatin (UGT1A3) Lamotrigine (UGT1A4) Valproic acid (UGT1A6)†† Zidovudine (UGT2B7)	Consider evaluation of variants of these genes to understand impact on PK and clinical outcomes
3	Newly discovered haplotypes	UGT1A1, UGT1A7 and UGT1A9	FOLFIRI (folinic acid, fluorouracil, irinotecan)‡‡	Consider evaluation of haplotypes as predictive markers of PK and/or clinical outcomes When multiple UGT1A enzymes are involved in drug clearance, association analyses of UGT1A haplotypes with drug clearance and clinical outcome may be more useful than analysis of individual SNPs. The utility of such information in confirming *in vitro* findings with regard to the role of UGT enzymes may be important.

†Data taken from [32].
‡Data taken from [33-37].
§Evaluation of possible DDI with *UGT1A1*28* is recommended in the USA FDA DDI Guidance.
¶Data taken from [38-40].
#Data taken from [7].
††Data taken from [33,41].
‡‡Data taken from [42,43].
CL: Clearance; DDI: Drug–drug interaction; PK: Pharmacokinetics; UGT: uridine diphosphate glucuronyltransferase.

and/or toxicity [78–80]. The c.421C>A variant in BCRP resulted in clinically significant increases in exposure of drugs such as rosuvastatin and sulfasalazine [81].

Similar to UGT, selective substrates and inhibitors for many transporters have not been identified for use in *in vitro* systems [53]. Selective substrates currently being used are summarized in Table 2. There are several reports on the *in vitro* selectivity of inhibitors for OATP-1B1, -1B3, -2B1, NTCP and OCT2 [82–84], but a systematic evaluation of utility of these inhibitors in phenotyping studies has not been conducted.

Human hepatocytes express the full complement of uptake transporters with protein levels of transporters similar to human liver [85–87]. Many transporters have broad and overlapping substrate specificities, making it challenging to de-convolute contributions of individual transporters to overall active transport. For example, with OATP-1B1, -1B3, -2B1 and NTCP, data between research groups on the relative contribution of each transporter to uptake have not been consistent [54,88–90].

Transporters in heterologous expression systems are often expressed at higher levels relative to *in vivo*, and therefore extrapolation factors based on activity (transport rates) or protein abundance for each transporter, similar to RAF or ISEF for enzymes, are needed to quantitatively model data obtained in these systems. In addition, transporter activity in recombinant systems can be affected by localization in the plasma membrane [91–93].

As an example, to determine the contribution of OATP-1B1 and -1B3 to hepatic uptake clearance, an RAF method (Equation 4, Table 1) was developed by comparing the uptake of test articles with relatively selective substrates of OATP-1B1 and -1B3 in recombinant cell lines and human hepatocytes. Alternatively, the relative expression factors (REFs) of OATP1B1 and -1B3 were estimated in the recombinant systems

Table 4. Effects of emerging relevant genetic polymorphisms of carboxylesterase 1 in Caucasians on the pharmacokinetics and pharmacodynamics of various drugs/substrates.

SNP	Drug	Ethnicity and number of individuals included in the trial	Clinical effect	Ref.
Carboxylesterase 1 (CES1)				
rs71647871 (c.428G>A, p.143Gly>Glu)	Oseltamivir	428 GG = 12 428 GA = 9 428 AA = 1	428 GA: 18% AUC increase, 428 AA: 360% AUC increase	[44]
	Methylphenidate	428 GG = 115 428 GA = 7	SNP carriers required 30% lower doses for symptom reduction in ADHD	[45]
	Clopidogrel	428 GG = 499 428 GA = 7	SNP carriers had 50% greater levels of clopidogrel active metabolite, and a better response as measured by ADP-stimulated platelet aggregation	[46]
rs3815583 (–75 T>G)	Methylphenidate	TT = 129, TG + GG = 76	G allele associated with worsening of appetite reduction in youths with ADHD	[47]
	Isoniazid	TT = 67, TG = 63, GG = 17	Associated with isoniazid-induced hepatotoxicity in patients with latent tuberculosis	[48]

ADHD: Attention-deficit/hyperactivity disorder; AUC: Area under the curve.

and human hepatocytes (Equation 5, Table 1), using Western blotting with OATP-specific antibodies, and recently, more quantitatively by LC–MS/MS technology [54,55,85,94]. In the cited references, use of RAF or REF approaches was considered successful to identify the contributions of OATP-1B1 and -1B3 to drug uptake. Alternatively, the contribution of OATP-1B1 and -1B3 to hepatic uptake can also be estimated by knocking down the expression of a particular transporter by siRNA probes [95,96]. Overall, experience with phenotyping methods for OATP1B and other transporters is still limited and more examples are needed to establish best practices.

Modeling strategies to predict the significance of PGx in patients

Using modeling and simulation tools is of great interest for companies to facilitate successful drug development. Important for these predictions is the *in vitro* reaction phenotyping to estimate the contributions of drug-metabolizing enzymes and transporters to drug disposition, as described above. Both static modeling, involving the use of general pharmacokinetic equations and assuming a constant drug exposure, as well as Simcyp® (Certara, NJ, USA)and GastroPlus® (Simulations Plus, Inc., CA, USA) dynamic modeling (where drug concentrations change over time) are equally utilized by 81% of 16 responders (Q13) to leverage *in vitro* data in support of Phase 1 clinical studies. DDI predict is reportedly used less often (25%) while none of the 16 respondents indicated the use of PK-Sim or other software packages at the time of the survey.

The success of modeling and simulation efforts by responding companies varied: five of 11 responders reported that their models built upon *in vitro* data were predictive within twofold when compared with the subsequent respective clinical data, while three responders each experienced either greater than twofold differences or within twofold differences for only some enzymes or transporters (Q16). Prediction accuracy is often judged on fold-error, targeting less than twofold difference relative to observed clinical data, although clinical sample size and between-study variation also play roles [97]. While not always considered quantitatively predictive, 12 of 14 companies indicated that preclinical strategies were sufficient to identify clinically important polymorphic enzymes or transporters (Q24). Failure to identify polymorphic enzymes or transporters was noted by six companies (Q25). Commentary from respondents included that predictions are generally more challenging for slowly

metabolized and poorly soluble compounds, or due to the lack of selective substrates and inhibitors for several enzymes or transporters, and that some enzymes and transporters are not routinely investigated in the commonly utilized *in vitro* systems. The interplay of absorption, the involvement of multiple enzymes and transporters in clearance, or extrahepatic metabolism and transport (when *in vitro* data are typically developed from hepatic models) requires more data and sophisticated models, which are typically not available in early clinical development, limiting the inclusion of PGx in clinical study plans.

The models used to predict the *in vivo* effects of genetic variants in enzymes and transporters from *in vitro* data can be broadly classified as either static or dynamic. Static models, not accounting for drug concentration–time profiles, are now well established and are relatively simple to construct. Indeed, this may be the preferred methodology in some instances, as suggested by a side-by-side comparison of predictions from static and dynamic modeling to describe the relationship between CYP2C19 polymorphisms and clopidogrel metabolism [98]. Generally, prior to Phase Ib, a static model can quickly be developed because data are often available. Nevertheless, a number of limitations exist for this kind of approach [99]. For instance, the impact of rare genetic variants, or more than one metabolism or disposition pathway with genetic variability can be difficult to model due to a paucity of data and lack of refinement of a model.

There are an increasing number of examples using dynamic or physiologically based pharmacokinetic (PBPK) modeling to predict and characterize the effects of genetic variants on PK. Using PBPK modeling, initial predictions of changes in exposure due to genetic polymorphisms are based on a 'bottom-up' approach where all drug-related inputs are derived from physicochemical characteristics, *in vitro*, and *in silico* data, usually combined with data from animals and/or interspecies extrapolation from animals to humans. Examples of the use of PBPK modeling are summarized in Table 5. In practice, at the end of Phase I, clinical data are available to further refine the initial PBPK model [100]. Incorporating clinical data in models is often referred to as a 'middle out' approach.

Compared to predicting the impact on PK of genetic variants in CYP or other enzymes, assessing the effect of transporter variants is more challenging. In addition to factors already noted, contributions to transport from the variant transporter, passive diffusion and other transporters must be estimated [53,108]. For example, a whole-body PBPK model was built for rosuvastatin incorporating the sinusoidal uptake transporters OATP-1B1, -1B3, -2B1 and NTCP, and the canalicular efflux transporter BCRP [105]. Hepatic CI_{int} for the three *OATP1B1* genotypes (c.521TT, TC and CC) was predicted by fitting the observed PK data using a 'top-down' approach. The results indicated that reduced OATP1B1 activity would result in relatively large increases in the area under the plasma concentration–time curve ($AUC_{0-infinity}$) of rosuvastatin (63 and 111% for the TC and CC genotypes, respectively, compared with the TT genotype). PD of rosuvastatin for specific *OATP1B1* genotypes driven by liver concentrations were modeled as part of this work. It is noted that the authors did not prospectively predict the impact of the OATP1B1 variants on the exposure or efficacy of rosuvastatin. In another PBPK paper, the authors predicted the impact of OATP1B1 variants on human PK for pravastatin and rosuvastatin using *in vitro* and clinical data for three major OATP1B1 genetic variants [107]. Based on the *in vitro* estimated fraction of OATP1B1 involvement in total hepatic active uptake and the ratio of uptake activities between variants, the authors concluded that the proposed PBPK modeling approach provided reasonably accurate predictions for individuals carrying specific allelic variants.

These examples show that PBPK modeling can be successful with transporters but requires incorporation of both *in vitro* and clinical data. Prediction of human transporter-mediated pharmacokinetics using 'bottom-up' static or dynamic physiologically based modeling approaches with only preclinical information has been proposed but is limited due to high uncertainty [109-111]. Successful models to date require unknown, but seemingly important, compound-dependent scaling factors [111].

Modeling of PGx-mediated differences in PK has also been attempted via population PK modeling approaches, although in late clinical development when sufficient clinical data are available. Application of population PK principles enables the attribution of the specific influence of genetic variants on the observed variability in PK parameters. Clearly, it is more of a 'top-down' approach through the assessment of covariate analysis on clearance and generally not suitable for prospective predictions on the effects of genetic variants on drug candidates [112,113].

PGx & other intrinsic & extrinsic factors of PK variability

When companies were asked for their general policy on genotyping Phase I clinical trial subjects, 65% responded that genotyping was done retrospectively, after high PK variability was observed in clinical trials (Q18). Genotyping based on *in vitro* prediction of polymorphic metabolism had been done by 24% of companies, and two companies (12%) routinely

Table 5. Application of physiologically based pharmacokinetic modeling to measure the effect of genetic variants on pharmacokinetics.

Enzyme/transporter	Drug	Ref.
CYP2C8	Rosiglitazone	[101]
CYP2C9	Warfarin	[102]
CYP2D6	Dextromethorphan	[103]
CYP-3A4 and -2D6	Aripiprazole, fesoterodine active metabolite 5-HMT, lloperidone, risperidone	[104]
OATP1B1	Rosuvastatin	[105]
OATP1B1	Pravastatin	[106]
OATP1B1	Pravastatin, rosuvastatin	[107]

5-HMT: 5-hydroxymethyl tolterodine.

genotype essentially all Phase I clinical trial subjects. However, 60% of companies indicated that *in vitro* data had influenced a decision to genotype subjects in the last 3 years, in at least 1–3 programs (Q20 and Q21). The combined list of genotyped targets encompassed many enzymes and transporters (Q22). One interpretation of these responses is that *in vitro* data typically drive decisions to genotype in Phase 1 studies if significant involvement of polymorphic enzymes or transporters is expected (e.g., meet or exceed thresholds in EMA guidance), and more often, that high PK variability observed in Phase 1 studies is an important consideration.

Related to this, Q23 was designed to survey rationales for conducting clinical studies with individuals of characterized genotype. Of the 17 responses to this question, 76% indicated that investigation of PK variability, while 41% indicated that inclusion or exclusion of individuals from a clinical study constituted the two main reasons for conducting PGx investigations.

In addition to PGx, multiple intrinsic and extrinsic factors can also influence the PK of a drug. These factors may include age, gender, race/ethnicity, disease state, smoking, diet, alcohol, concomitant medications, organ dysfunctions and other physiologic changes such as pregnancy [114]. Recent evidence also suggests that epigenetic mechanisms (e.g., histone modification, DNA methylation and noncoding RNAs) regulating ADME-related genes potentially contribute to PK variability in humans [115]. Typically, many of these intrinsic and extrinsic factors are identified in the mid- to late phases of drug development and are not available to help formulate a PGx plan in early clinical development. As stated in the FDA PGx Guidance, an understanding of specific covariates and gene-covariate interactions on variability in drug response could be useful in determining the relative impact of genetics, versus other nongenetic factors, on the PK, PD, dosing, efficacy and safety of the drug. Therefore, multivariate consideration is essential to fully evaluate and understand how the genetic and nongenetic factors may contribute to the overall variability of a drug. For instance, in addition to the status of *CYP2D6* genotype, the dosing recommendation of atomoxetine for the treatment of ADHD in children and adolescents also takes body weight into consideration [4]. In the case of warfarin, dosing algorithms that include intrinsic and extrinsic factors such as age, height, weight, sex, ethnicity, smoking status and interacting drugs in addition to *CYP2C9* and *VKORC1* genetic polymorphisms provide an improved prediction of the optimal dose to achieve the target international normalized ratio [116,117]. These examples illustrate the multifactorial nature of PK variability, among which PGx may be one factor.

Relevance of genetic polymorphism data to a discovery and development program is usually interpreted with considerations for disease indication and/or frequently coadministered drugs. Based on the responses to Q19 of the survey, 64–93% of the 14 responses indicated that disease indications (oncology and nononcology), as well as coadministered drugs for the disease indications, are considered when interpreting the PGx data. This appears to be consistent with the responses to Q17, in which two of 16 and 12 of 16 respondents said that they had no decision criteria, or acted case-by-case, respectively, to progress a drug candidate based on predicted involvement of polymorphic enzymes or transporters in drug disposition. While the survey did not address the specific considerations involved in such decisions, it may be that factors such as urgency of unmet medical needs, predicted therapeutic index, generation of active metabolite(s) that are impacted by genetic variation (e.g., codeine and tamoxifen) play an important role in the decision-making process. Responses to Q17 and Q19 reflect the complex considerations involved when applying PGx science to drug discovery and development programs.

The survey also indicated that investigation of efficacy (12%) or safety (29%) were less common reasons for assessing the impact of PGx. This was not unexpected since the focus of the survey was in early drug development. Small sample size in Phase 1 studies, differences in study population (commonly healthy volunteers of relatively homogeneous population, instead of the intended patient population) and lack of efficacy and/or safety assessment may preclude meaningful evaluation of genetic variants on efficacy or safety in early drug development. However, the examples illustrated in the FDA and EMA Guidance show the focus of regulatory review regarding the contribution of PGx on efficacy (e.g., clopidogrel and CYP2C19), safety (statins and SLCO1B1) and selection of optimal dosing (e.g., warfarin and CYP2C9 and VKORC1), in addition to PK variability. These aspects remain subjects of research interest and may serve as possible topics for future follow-up. This is covered in more detail in a recent paper from the I-PWG [118].

Discussion

Ideally, incorporating ADME PGx into clinical development plans will begin prior to clinical testing, by utilizing data from *in vitro* systems. Most companies responding to the survey indicated that they routinely use *in vitro* systems to investigate involvement of various ADME-related enzymes and transporters in disposition of new compounds prior to beginning clinical studies. While the primary reason for this may be to elucidate metabolism or transport (distribution) of compounds, or potential drug interactions during discovery, this information is also used to consider whether enzyme or transporter genetic variants will contribute to variability in drug disposition, PK and PD.

In vitro systems for the major CYP enzymes are generally available and well understood for their strengths and weaknesses. Experience is growing for other enzymes and transporters, although availability of useful *in vitro* systems is limited. Although qualitative information of involvement of a polymorphic enzyme or transporter in disposition of new chemical entities is useful in early drug discovery, the goal is to develop quantitative models as early as possible. The majority of companies are using *in vitro* systems to test for the better understood enzymes and transporters, but the number of companies testing for less understood proteins drops dramatically, due to lack of availability or significance observed to date of genetic variants that impact PK. Thus, the number of enzymes and transporters typically tested preclinically is not as extensive as the regulatory guidelines suggest. In addition, companies may take into account that the level of tolerance for variability in PK must be interpreted in the context of the treatment indication and therapeutic window [119].

Similar to *in vitro* systems, *in silico* models incorporating *in vitro* and animal data are being routinely used and are improving. Many companies reported successful use of models to understand genetic sources of variability, at least within twofold of observed clinical data. Unfortunately, there are still many examples where models are not quantitatively predictive, for various reasons highlighted in this paper. With continuing improvements (e.g., identification of selective substrates and inhibitors, quantification of enzyme and transporter protein amounts, and improved *in silico* models), we expect an increase in utilization of *in vitro* data to incorporate PGx in early clinical plans.

Although use of *in vitro* data to guide development of early clinical PGx plans is strongly suggested by the EMA and FDA Guidances, the majority of companies do not prospectively genotype in Phase I. Instead, when high variability is observed in early clinical studies, retrospective genotyping is conducted to investigate PGx as a source of that variability. Overall, the survey indicates that companies are utilizing *in vitro* data, when there is some confidence in predictability, when estimated f_m or f_t values for a polymorphic enzyme or transporter are high (≥50%), or after some clinical experience.

More attention is needed to developing more and better *in vitro* systems and *in silico* models. For example, the functional impact, if any, of variants in many ADME genes is not understood or there may be rare but important variants yet to be identified. The time and expense of increasing the number of enzymes and transporters tested *in vitro*, and their variants when available, must be considered in the evolving industry strategy to move quickly to clinical proof-of-concept studies, while delaying experiments that have typically been conducted early in development. Dependability of *in silico* models continues to improve, but significant resources are required to develop more complex, and hopefully more predictive, models. Such models may not be achievable preclinically or in early clinical development.

Conclusion

It is clearly the goal of pharmaceutical companies and regulatory agencies to understand the complex interplay of genetic variants of enzymes and transporters on drug disposition. However, it is important to remember that genetic variation is only one of many sources of drug PK and PD variability. As *in vitro* systems improve and modeling becomes more predictive, sources of variability will be better understood and evaluation of

impact on PK and PD will be incorporated earlier into drug development.

The examples most cited today of the use of ADME PGx in drug development and disease treatment (e.g., warfarin and clopidogrel) were identified after marketing and extensive use of the drugs. Ideally, potential ADME-related PGx issues would be identified during (early) development, so this is well understood at the time of new drug approval. Industry and regulatory efforts are making progress toward this goal.

Future perspective

Understanding of genetic variation of drug metabolism enzymes and transporters, including functional consequences, continues to increase. The major CYP enzymes are fairly well understood but more needs to be learned for other enzymes and transporters. Although *in vitro* experimental systems to investigate these proteins are extensively used in pharma, advancements are needed. Availability of additional enzyme and transporter systems should increase, including those with variant proteins. Selective substrates and inhibitors for some proteins, such as UGT, and many transporters must be identified. More sophisticated tools are being developed, such as *in vitro* 3D microfluidic systems and humanized animals. Modeling software is becoming more sophisticated and also more widely available. Experience with this software will continue to grow. Coupled with high-quality *in vitro* data, the predicted impact of genetic variation will be better understood as one of many intrinsic and extrinsic factors contributing to drug PK and PD. This will allow incorporation of pharmacogenetic evaluation earlier in clinical develop-

Executive summary

Introduction

- The Industry Pharmacogenomics Working Group surveyed member pharmaceutical companies on the use of *in vitro* systems and *in silico* models to predict the impact of genetic variants in drug metabolism enzymes and transporters on clinical development of new drugs.

Phenotyping drug metabolism enzymes & transporters *in vitro*

- Prior to Phase I clinical trials, *in vitro* phenotyping studies are conducted by 88% of companies to test for potential involvement of drug metabolism enzymes and transporters in drug disposition.
- Genetic variants in CYP enzymes considered very relevant across companies are CYP-2C8, -2C9, -2C19, -2D6 and -3A.
- Uridine diphosphate glucuronyltransferase (UGT) enzymes are studied preclinically by about half of the companies surveyed with UGT1A1 more commonly studied than others.
- Most companies investigate the role of P-glycoprotein (MDR1), OATP1B1 and BCRP prior to Phase I, whereas other drug transporters are analyzed on a case-by-case basis.
- *In vitro* phenotyping methodology and clinical translation is relatively mature for CYP enzymes but remains an area of continued research for other enzymes and transporters.

Modeling strategies to predict the significance of pharmacogenetics in patients

- Modeling and simulation of the effects of genetic variants of enzymes and transporters is utilized in the industry to predict the clinical impact on pharmacokinetics (PK) and pharmacodynamics (PD) in the clinic.
- Accurate prediction based on *in vitro* data only, before clinical data are available, is not always successful due to limitations of *in vitro* systems or unknown factors required for modeling.

Pharmacogenetics & other intrinsic & extrinsic factors of PK variability

- Most companies do not prospectively genotype drug metabolism enzymes and transporters in Phase I clinical trials. Genotyping is more often driven by observations of high pharmacokinetic variability in Phase I trial subjects, rather than prediction based on *in vitro* systems and modeling.
- The most common primary rationale to conduct clinical studies with individuals with characterized genotype was to investigate genetic variation as a source of pharmacokinetic variability.
- Decision criteria regarding drug candidate progression based on predicted involvement of a polymorphic enzyme or transporter in drug disposition often is case-by-case.
- Interpreting the relevance of genetic variation for a discovery or development program commonly takes into consideration the disease indication and/or frequently coadministered drugs.

Discussion

- There are multiple sources of PK and PD variability, of which genetic variation is one component.
- Continued improvements of *in vitro* systems and *in silico* modeling are needed to improve prediction of the significance of genetic variation in drug metabolism enzymes and transporters on PK and PD variability prior to, or early in, clinical development.
- The goal of the pharmaceutical industry and regulators is to understand the impact of genetic variation as early as possible in drug development to optimize efficacy and safety of new drugs.

ment, as described in current regulatory guidances. However, even as the availability and quality of *in vitro* systems and models increase, there still will be challenges with evaluating a large number of different enzymes and transporters in discovery or preclinical development. The focus may stay limited to a relatively narrow set of enzymes and transporters, where functional consequences have been observed. Evaluation of less common genetic variants, particularly without known functional or clinical significance, may not be commonly embraced. Without clear indication of a variant enzyme or transporter being significantly involved in drug disposition, companies may decide to risk unknown genetic variability in the clinic, rather than utilizing an extensive *in vitro* program prior to entering clinical development. The challenge to industry and regulators will be to better predict the impact of genetic variation in enzymes and transporters while developing new drugs, rather than after marketing, where most of today's examples are known.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/pgs.16.9

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