REVIEW ARTICLE

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Predicting the Clinical Relevance of Drug Interactions From Pre-Approval Studies

Silvio Caccia,[1] *Silvio Garattini*,[2] *Luca Pasina*[3] and *Alessandro Nobili*[3]

1 Laboratory of Drug Metabolism, 'Mario Negri' Institute for Pharmacological Research, Milan, Italy
2 'Mario Negri' Institute for Pharmacological Research, Milan, Italy
3 Laboratory of Quality Assessment of Geriatric Therapies and Services, 'Mario Negri' Institute for Pharmacological Research, Milan, Italy

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Abstract

Drug interactions (DIs) may result in adverse drug events that could be prevented, but in many cases the available information on potential DIs is not easily transferable to clinical practice. The majority of studies date from preclinical or premarketing phases, using animals or human-derived sources that may not accurately reflect the growing clinical complexity of high-risk populations, such as the elderly, women, children, patients with chronic disease, polytherapy and impaired organ functions. Thus, at the time of approval of a new drug the information in the summary of product characteristics refers to potential DIs, but lacks specific management recommendations and is of limited clinical utility.

Therefore, we set out to review *in vitro* and *in vivo* methods to predict and quantify potential DIs, to see whether these studies could help the physician tackle daily problems of the assessment and choice of combined drug therapies,

and to propose, from a clinical point of view, how premarketing studies could be improved so as to help the physician at the patient's bedside.

Preclinical and premarketing study design needs to be improved to make information easily accessible and clinically transferable. Studies should also take into account appropriate sample size, duration, co-morbidity, number of coadministered drugs, within- and between-subject variability, specific at-risk populations and/or drugs with a relatively narrow therapeutic window, and clinical endpoints. After premarketing development in situations where there is potential high risk of serious adverse events, specific phase IV studies (and/or active pharmacovigilance studies) should be required to monitor and quantitatively assess their clinical impact.

Drug interactions (DIs) are generally undesired pharmacological responses that may be prevented or avoided, but in many cases information is incomplete and difficult – even impossible – to translate into true clinical risk and appropriate action. Serious adverse events related to pharmacokinetic or pharmacodynamic DIs often come to light only after the approval of a new drug, even though its safety has been appropriately evaluated in premarketing studies;[1-4] in the last decade (table I) several drugs, such as astemizole,[5,6] cerivastatin,[7,8] cisapride,[9] mibefradil[10,11] and terfenadine,[12] have been taken off the market because of serious DIs.

According to several studies, DIs and related adverse drug reactions (ADRs) are important causes of morbidity and mortality,[13-16] and impose a significant burden in terms of healthcare costs.[17-19] This is why an evaluation of potentially serious DIs is now mandatory for the approval of new drugs by international regulatory agencies, some of which have developed specific guidelines[20-23] that primarily require studies on inhibition and induction of drug metabolizing enzymes. Unfortunately, the majority of these studies are done in preclinical settings using animals or human-derived sources, which may not accurately reflect the complexity of patients and the settings in which the drug will be used in practice; healthy, young, generally male volunteers are usually considered with advanced drug candidates, later in the development pipeline.[2,3,24-27] DI studies are mainly aimed at providing information that will be included in the summary of product characteristics (SPC) to avoid liability.[3,28] More importantly, most of these studies use the 'drug' as the main outcome instead of using patient outcomes.[29,30] Thus, at the time of

Table I. Examples of drugs withdrawn from the market because of interactions with other drugs

Drug	Therapeutic use	Date withdrawn	Primary safety risk
Mibefradil	Angina and hypertension	1998	HMG-CoA reductase inhibitor ('statin')-induced rhabdomyolysis QTc prolongation[a] and TdP
Terfenadine	Allergic rhinitis and urticaria	1998	QTc prolongation[a] and TdP
Astemizole	Allergic rhinitis and urticaria	1999	QTc prolongation and TdP
Cisapride	Gastroesophageal reflux disease	2000	QTc prolongation[a] and TdP
Levacetylmethadol	Opiate dependence and severe pain	2001	QTc prolongation[a] and TdP
Cerivastatin	Hypercholesterolaemia and mixed dyslipidaemia	2001	Statin-induced rhabdomyolysis
Dofetilide	Atrial fibrillation and atrial flutter	2004	QTc prolongation and TdP

a Evidence of greater risk in women.

QTc = corrected QT interval; **TdP** = torsades de pointes.

registration most of the data on DIs cannot be easily translated into clinical practice.[31-35]

Therefore, the aims of this review are (i) to examine the *in vitro* and *in vivo* methods used to predict and quantify potential DIs, comparing their limits and potentials; (ii) to see whether these studies can help physicians in their clinical activity; and (iii) to suggest, from a clinical point of view, how premarketing DI studies could be improved to help the clinician at the patient's bedside.

1. Predictive Assay for Drug Interactions (DIs)

As mentioned, DIs can often be explained by inhibition or, perhaps to a lesser degree, induction of hepatic and extra-hepatic cytochrome P450 (CYP) enzymes, particularly members of the subfamilies 1A, 2C, 2D and 3A, which metabolize a broad spectrum of drugs by a number of metabolic processes. These isoenzymes are particularly prone to inhibition because of their broad substrate spectrum, which allows their substrates to be competitively inhibited by structurally different xenobiotics biotransformed by the same enzymes. Some CYP enzymes are highly inducible by drugs, pollutants, cigarette smoking, alcohol and dietary constituents.[36] Inductive and inhibitory interactions, however, may also involve oxidative systems different from CYP and also phase II superfamilies of isoenzymes and drug transporters, although less frequently than the CYP-mediated interactions.[37,38] Lists of inducers and inhibitors of drug-metabolizing enzymes and drug transporters are regularly updated at several internet sites: http://medicine.iupui.edu/flockhart/ or http://www.nihs.go.jp/mpj/interact.htm; see also the FDA[22] and EMEA[23] guides for DI studies.

Enzyme inhibition and enzyme induction are of particular clinical significance when they involve drugs with a relatively narrow therapeutic window or drugs chronically and extensively used by 'at high-risk populations', such as children and elderly patients, particularly if they are exposed to polypharmacy. An important concern is that inhibition- and induction-mediated interactions may be a significant cause of inter- and intraindividual variability to a particular therapy, which constitutes the basis for many cases of 'failure' or 'exaggerated response' in general healthcare. Therefore, understanding and anticipating serious interactions is a necessary part of rational therapeutics. Hence, the efforts of pharmaceutical research to identify the enzymes and genetic variants responsible for biotransformation of new chemical entities during their discovery and development processes, are aimed at predicting potential interactions with concomitantly administered substrates, inhibitors and inducers of the same enzymes.

DIs can also affect gastrointestinal absorption, distribution (plasma and/or tissue protein binding) or excretion. Pharmacodynamic changes that can result in DIs include competition for a receptor site, alteration of a receptor at the site of action, and additive or opposite effects of a drug on other biological systems.

1.1 Current Tools

This section summarizes what pharmaceutical companies actually do, and are required to do by regulatory agencies, to address metabolically based DIs in drug development. A brief summary of methods currently in development, that may potentially help to bridge the gap in differences between animal and human tools through understanding of biological mechanisms, is also included. The relative advantages and disadvantages of these procedures are compared in table II.

1.1.1 Studies in Animal Models

Although pharmaceutical companies still use *in vitro* and *in vivo* animal models to understand the mechanism(s) of drug metabolism and DIs in animal systems and, through this understanding of the mechanism, attempt to make an assessment of whether the behaviour is expected to translate to humans, it is now generally accepted that these studies can be misleading and need to be interpreted with great caution. This is because metabolic differences between humans and animals make extrapolation to the clinical setting

Table II. Examples of *in vitro* and *in vivo* models for predictive drug interaction (DI) studies

Model	Main scope of use	Limitations
Current tools		
Animal models	Demonstrating species differences in drug metabolism; understanding the mechanism(s) of DIs	Species differences in CYP isoenzymes and their relative abundance. Species differences in nuclear receptor activation
Human liver microsomes	Predicting drug metabolic clearance Screening of CYP inhibition properties	Loss of functions over time Very low phase II activities Process missing in subcellular models not accounted for
Fresh, cryopreserved and fetal primary hepatocytes	Screening of CYP inhibition properties Global examination of inductive potential	Poor availability of fresh cells, variability in response, gradual decrease in CYP expression. Basal activity of some CYP is low (cryopreserved cells) or CYP profile and regulation do not match the adult situation (fetal hepatocytes)
In vivo studies in healthy subjects	General pharmacokinetics. To confirm initial *in vitro* DI data	Costly, time-consuming; result might not reflect the situation in populations at particular risk for specific interactions
Tools in development		
Continuous, immortalized and stem cell lines	High-throughput screening for lead optimization	Major CYP activities low, if detectable at all in some cell lines; lack of activation of CAR and low or absent expression of some hepatic uptake transporters in others
Reporter-gene assay	Predictive identification of CYP gene transcriptional inducers	Potential mechanisms other than receptor-mediated activation not accounted for
Liver slices	Metabolite measurements	Survival of cells within the sliced tissue. Not yet adequately established for DI prediction, although it is already apparent that the extent of induction is often lower than in primary hepatocytes
Humanized animal models	Predicting DIs triggered by human nuclear receptor ligands	Availability. Potential effects of other rodent factors relevant for drug metabolism and species differences on the receptor's target gene repertoire
In silico methods	Predicting substrates and inhibitors of CYP enzymes as well as ligands of nuclear receptors	The software packages for prediction of DIs have not yet been properly validated and still require improvement

CAR = constitutive androstane receptor; **CYP** = cytochrome P450.

very difficult. This is largely the consequence of the different CYP enzymes expressed in humans and other species, and their relative abundance. For example, while CYP1A2 is the predominant CYP1A form in all species, CYP3A4 and CYP3A1 are the CYP3A isoforms preferentially expressed in humans and rats respectively; similarly, CYP2C9 predominates in humans, CYP2C11 in the rat and other isoforms in the mouse, rabbit, pig and dog among the members of the CYP2C subfamily[39,40] (see also table III).

The CYP2D subfamily has at least nine active genes in the mouse, five in the rat but only a single functional isoform in humans, CYP2D6, which is responsible for the metabolism of approximately one quarter of all clinically used drugs[41] and is linked to a well known polymorphism with almost 100 defined allelic variants, many of which are associated with increased, decreased or abolished function of the final gene product.[42] The rat and human CYP2D catalyses hydroxylation of debrisoquine, which is competitively inhibited by quinidine and its diastereoisomer quinine. However, *in vitro* quinidine is a more potent competitive inhibitor of this reaction in humans than in rats, while the reverse is true for quinine, which indicates that data on the specificity of CYP2D in the rat must be extrapolated to humans with extreme caution.[43] Moreover, different strains of mice may have a low capacity to metabolize debrisoquine.[44] A transgenic mouse line expressing the human CYP2D6 gene has been recently generated, where the transgenic and wild-type mouse lines act as models of human extensive metabolizers (EM) and poor metabolizers (PM), respectively. Unlike wild-type mice, these humanized

mice display significant debrisoquine-4-hydroxylase activity and a pharmacokinetic profile similar to humans. Pending further evaluation, this preclinical model may be of considerable interest for the preclinical evaluation of novel CYP2D6 substrates and inhibitors, and identification of *in vivo* DIs.[45]

As mentioned, induction-mediated interactions are one of the major concerns in clinical practice and for the pharmaceutical industry because induction may increase the clearance of other drugs, resulting in a decrease in therapeutic efficacy. Induction may also increase in the activation of prodrugs with alterations to their efficacy and pharmacokinetics and an increase in the bioactivation of drugs that contribute to hepatotoxicity via reactive intermediates.[46-49] For example, many CYP3A4 inducers are used as drugs in clinical practice and they can induce CYP3A4, which is responsible for the biotransformation of the majority of drugs currently on the market. Thus, oral midazolam, simvastatin and most L-type calcium channel antagonists are ineffective during rifampicin (rifampin) treatment.[50] Similarly, the induction of the metabolism of ciclosporin (cyclosporine) by rifampicin resulted in organ graft rejection, whereas the herbal medicine *Hypericum perforatum* (St John's wort), possibly through its main phloroglucinol component hyperforin,[51] attenuates the efficacy of several drugs on the market at present.[52,53] Therefore, screening drug candidates for induction potencies is important in premarketing studies. Unfortunately, animal CYP induction studies may not always be good models of CYP induction in humans. For example, pregnolone-16α-carbonitrile *in vitro* potently induces CYP3A in rat but not in human hepatocytes.[54] As recently reviewed by Hewitt et al.,[47] there are also marked species differences in the CYP1A, CYP2B and CYP2C induction responses.

The mechanism by which CYP3A4 is upregulated involves intracellular binding of the inducer to the nuclear pregnane X receptor (PXR). Subsequently, this receptor heterodimerizes with the receptor for 9-cis retinoic acid (RXR). The heterodimer then functions as a transcription factor by interacting with cognate response elements located in the 5′ regulatory region of the CYP3A4 gene; the net result is increased synthesis of new CYP3A4 protein.[55,56]

Although mouse, rat, rabbit and human PXR are activated by several common CYP3A substrates, the ligand specificity of these receptors is species specific and this results in differences in the response to various inducers.[36,57,58] Thus,

Table III. Main drug-metabolizing cytochrome P450 (CYP) enzymes in human and other species[a]

Subfamily	Human[b]	Preclinical safety species			
		monkey[c,d]	dog	rat	mouse
CYP1A	1, 2	1,[c,d] 2[c,d]	1, 2	1, 2	1, 2
CYP2A	6, 7, 13	23–24[d]	13, 25	1,[e] 2,[e] 3	4,[e] 5, 12, 22
CYP2B	6	17[c]	11	1–3, 8, 12, 15, 21	9–10, 13, 19, 23
CYP2C	8, 9, 18, 19	20,[c] 74–75,[d] 76[b]	21, 41	6–7,[e] 11–13,[e] 22–24, 46	29, 37–40, 44, 50, 54, 55, 65–70
CYP2D	6	1, 7[c] 42[d]	15	1–5, 18	9–13, 22, 26, 34, 40
CYP2E	1	1[c,d]	1	1	1
CYP3A	4, 5, 7, 43[f]	5,[c] 8,[c] 64,[d] 66[d]	12, 26	1–2,[e] 9,[e] 18,[e] 23, 62	11, 13, 16, 25, 41, 44, 57, 59

a Adapted from Donato and Castell[39] and Martignoni et al.,[40] and Nelson homepage: http://drnelson.utmem.edu/P450.stats.all.htm

b Pseudogenes are not listed.

c Cynomolgus monkey (*Macaca fasicularis*).

d Rhesus monkey (*Macaca mulatta*).

e Sex differences.

f The functional significance of this gene has only recently been demonstrated.

rifampicin strongly activates human PXR but is a weak activator of mouse and rat PXR. In contrast, pregnolone-16α-carbonitrile and dexamethasone strongly activate rodent PXR but are weak activators of human PXR. This may explain why animal models have often failed to predict the inductive potential of drugs in humans.

Other genes have PXR-RXR response elements in their 5′ regulatory regions. Consequently, rifampicin upregulates other CYP enzymes, including CYP2C9,[59] as well as other proteins that govern the absorption, distribution and excretion of drugs.[60] Nuclear receptors such as the constitutive androstane receptor (CAR) also seem to play a role in the induction of CYP3A4 and other CYP enzymes. As with PXR, human, rabbit and rodent CAR are all roughly equally divergent and share only ~70% amino acid identity, markedly differing in their responses to xenobiotics. This is the case for clotrimazole, which is an efficacious deactivator of human CAR in humans but has little or no activity on mouse CAR. Conversely, the planar hydrocarbon 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene is a potent activator of mouse CAR but lacks any such activity on human CAR. The divergence in amino acid sequences across CAR orthologs undoubtedly contributes to species differences in the physiological effects of xenobiotics.[61]

1.1.2 In Vitro Human Models

Because species differences in nuclear receptor activation make it problematic to predict CYP induction in humans from animal models at present, *in vitro* human models have become widely used to evaluate potential DIs.[62,63] These include enzyme-based systems (liver microsomes and complementary DNA [cDNA]-expressed enzyme systems) and cell-based systems (e.g. hepatocytes, hepatoma cell lines and liver slices). The human liver microsomal system has been found to be the most feasible and well set-up for quantitative or semi-quantitative prediction of *in vivo* drug inhibition, resulting in reversible inhibition mechanisms. However, there are some added complexities in predicting DIs from irreversible (often referring to mechanism-based) inactivation data.[64]

Several factors can significantly affect the results of studies in liver microsomes, limiting predictivity for the situation in a patient. The basic notion for *in vitro* inhibition studies is to use a selective CYP probe substrate and measure inhibition of metabolism in the presence of a second drug. These studies rely on the reaction adhering to steady-state assumptions (e.g. Michaelis-Menten kinetics) to determine the inhibitory constant (K_i) for the inhibitor accurately.[65,66] Unfortunately, establishing whether a new drug candidate is a potent CYP inhibitor is not straightforward and results may be ambiguous. As reviewed elsewhere,[25,27,62,67] drug inhibition of CYP enzymes can be affected by factors within the *in vitro* environment (e.g. non-specific binding of substrates to the incubation matrices, incubation conditions that deplete the unbound concentration of inhibitor available to the enzyme, etc.) or substrate-dependent factors (e.g. inhibition data, particularly for many CYP3A4 substrates, cannot be described by the Michaelis-Menten model, which may introduce serious errors into a quantitative interaction prediction based on the K_i value obtained *in vitro*).

In addition, the actual concentrations of substrate and inhibitor available to the CYP enzyme may depend on processes that are lacking in subcellular models (e.g. transport mechanisms, cytosolic enzymes, binding to intracellular proteins). With CYP3A4 substrates, for example, an interaction may be underestimated because *in vivo* there may be some contribution from DIs in the gut wall, which is a site of expression of CYP3A and multiple transporters. An example of this is the human multidrug-resistance (MDR) gene product permeability-glycoprotein (Pgp), which can limit the amount of PXR ligand and, therefore, the extent of CYP3A induction.[68]

A potential metabolically based interaction may also affect the formation of an active metabolite, which *in vivo* may present intra- and inter-individual differences in its pharmacokinetics, as exemplified by some selective serotonin reuptake inhibitors and other new-generation antidepressants,[69] but the *in vitro* system and conditions may not detect the phenomenon. Thus, the CYP2D6 inhibitor haloperidol raised

exposure to the active 1-(3-clorophenyl)-piperazine (mClPP) in depressed patients taking trazodone.[70] Similarly, the addition of fluoxetine to trazodone in depressed patients and to nefazodone in healthy volunteers raised plasma concentrations of their common metabolite mClPP. The effect persisted for several days because of the long elimination half-life of seproxetine, which is equally active to the parent drug in inhibiting CYP2D6 (but more potent as an inhibitor of CYP3A).[69] Fluoxetine, but not low doses of haloperidol, also raised plasma concentrations of trazodone (possibly because its biotransformation is partly mediated by CYP2D6) but did not affect plasma concentrations of nefazodone. Likewise, repeated doses of deramciclane, a novel anxiolytic with CYP2D6 inhibitor activity, had no effect on the clearance of buspirone (another anxiolytic drug, which is mainly metabolized by CYP3A4) but inhibited the further CYP2D6-dependent metabolism of its active metabolite 1-(2-pyrimidinyl)-piperazine.[70] Therefore, at present, the information gained from these *in vitro* studies is useful in drug screening[71] but their reliability in quantitative prediction for clinically relevant interactions depends on accurate *in vitro* data and knowledge of the drug pharmacokinetics and metabolism within and between populations.[72]

Prediction can be improved by combining *in vitro* metabolism data with appropriate modelling and simulation tools. One such tool is Monte Carlo simulation whereby *in vitro* metabolic results are incorporated into general pharmacokinetic and demographic models.[62] SIMCYP®[73] can also potentially predict clearance for drugs that are primarily metabolized by CYP and the magnitude of any DIs that may arise with other drugs by using data from human *in vitro* experiments. In addition, the software can be used to optimize the design of a clinical trial to ensure that any interaction is measured properly. By measuring substrate loss in human liver microsomes in the presence and absence of specific chemical inhibitors and in individual cDNA-expressed CYPs (recombinant human CYP [rhCYP]) it was recently shown that the use of rhCYP with SIMCYP® provides a more robust *in vitro* measure of CYP's contribution than chemical inhibitors combined with human liver microsomes for the prediction of potential CYP3A4 DIs before clinical investigations are started.[74]

Screening for potential inducers cannot be done in microsomes as it requires a cellular system fully capable of transcribing and translating CYP genes.[75] Because it is increasingly understood that inductive DIs are more common than previously thought, the US FDA has recently issued draft guidance[76] on models to predict the ability of drugs to induce hepatic CYP enzymes *in vivo*.

However, primary hepatocytes have scarce and unpredictable availability, limited growth and lose their enzymatic activity very quickly. Several approaches have been tested to improve the preservation of liver-specific functions, and marked phenotypic changes have been noted, resulting, particularly, in reduced expression of several major CYP forms.[77] Huge differences in the basal level of expression of CYP genes, as well as in the magnitude of enzyme induction after prototypical inducers, have also been reported from one human hepatocyte population to another, which reduces the predictive reliability of this method. The impact of cell culture conditions on the restoration and maintenance of normal hepatic structure and function can also lead to considerable variability, as reviewed by LeCluyse.[78] Moreover, this and other *in vitro* models cannot evaluate the risk to individuals, and interpreting results requires information on the observed and theoretically conceivable extreme effects in individual patients in the various populations.[62]

Recently, chimeric mice have been developed with liver humanized by the transplantation of human hepatocytes. Dexamethasone 6-hydroxylase (a human activity probe for CYP3A4) was increased in the liver microsomes of these mice after rifampicin dosing. Other CYP enzymes were also induced, suggesting that this model could be applied for *in vitro* and *in vivo* induction studies. However, these chimeric mice have several major disadvantages that have prevented their widespread use, including poor breeding efficiency, a narrow time window for transplantation and renal disease in repopulated mice.[79]

A more robust model of humanizing mouse liver was recently described by Azuma et al.[80] After pretreatment with a urokinase-expressing adenovirus, these mice could be engrafted (up to 90%) with human hepatocytes from multiple sources, including liver biopsies. Like the human hepatocytes, these chimeric animals show a normal response to CYP induction by inductive drugs, and express hepatic nuclear receptor transcription factors, conjugation enzymes and major transport proteins, thus offering the possibility of assessing the role of these gene products in human drug metabolism *in vivo*.

1.1.3 Studies in 'Healthy' Volunteers

At later stages of drug development, the aim of DI studies is to predict as accurately as possible the clinical consequences of potential interactions suspected from animal data or expected from human *in vitro* studies. However, while proper study design, clear targets and populations certainly improve the ability to distinguish detectable interactions from clinically relevant interactions (i.e. the drug activity and/or toxicity is modified to such an extent that changes in dose and/or frequency of administration are required), these studies are often done in limited numbers of healthy, generally male, adult volunteers, who receive only the two drugs involved. Women are often excluded from these studies, as they are from research studies in general, essentially because of well intentioned but ultimately imprudent efforts at safeguarding this population.[81-85] However, this exclusion limits the ability to predict clinically relevant DIs in approximately 51% of the population (54% of them of childbearing age) because of sex differences in drug pharmacokinetics and pharmacodynamics.[86] For example, females weigh less than males, although often no dosing recommendations are made on the basis of sex. For drugs in the bioequivalence studies recently evaluated by the FDA, not adjusting for weight resulted in 20–88% higher exposure in females than in males where there was a significant sex difference.[87]

Women have lower activity than men for CYP1A2 and CYP2E1, and there is also evidence of sex-related differences in the activity of CYP2C19 that appear ethnically dependent.[85] Also, females have higher CYP3A4 activity than males, with many CYP3A4 substrates showing slightly higher clearance in women after intravenous dosing, even after correcting for body weight.[88,89] Sex-related differences also exist in some mechanisms involved in drug elimination and distribution,[85,86] including membrane transport systems.[90]

The elderly, who comprise approximately 18–20% of the total population, are also usually excluded from DI studies because of their physiological and pathological differences from the younger adult population.[91] However, since they so often have multiple co-morbidities, the elderly too are at special risk of DIs, and also because they consume 45–60% of all prescriptions and use more than 40% of over-the-counter drugs.[18,92-95] It is estimated that drug-metabolizing capacity is reduced by approximately 30% after the age of 70 years.[96] A recent review of literature suggested age-related decreases in clearance of approximately 25% for CYP2C9 and CYP2C19 substrates and 20% for CYP2D6 substrates compared with younger patients, with slower clearance in women than men. Although age does not significantly affect CYP3A substrate clearance in cross-sectional studies of clinical populations, polytherapy may have greater effects on the clearance of CYP3A substrates than other environmental factors and must be considered in optimizing any dosing regimen.[86]

Glomerular filtration rate and renal function both decline with age. Drug distribution may be altered in the elderly, with serum albumin levels decreasing and α_1-acid glycoprotein rising with age.[86,97] Studies in healthy adult volunteers, therefore, provide only rough predictions and guidance, and DIs of minor clinical importance found in this 'healthy' population might become serious in the clinical setting. This was demonstrated impressively with the withdrawals from the market of the calcium channel antagonist mibefradil and the HMG-CoA reductase inhibitor ('statin') cerivastatin shortly after they had been approved in Europe and the US.[7,8,98,99]

In the limited population studied prior to marketing, mibefradil had appeared as efficacious

and safe as other calcium channel blocking agents.[100,101] It was known that this drug inhibited CYP1A2, 2D6 and 3A4, and the package insert specified that it was contraindicated with astemizole, cisapride, and terfenadine on account of increased risk of QTc prolongation.[10] However, an unusual number of new drug contraindications were discovered after its introduction, mainly related to the inhibition of CYP3A4-mediated biotransformation of commonly used drugs, which are most likely to occur in the elderly and in patients with baseline bradycardia. Cases of life-threatening cardiogenic shock have been reported in patients taking mibefradil and β-adrenoceptor antagonists, who also began taking dihydropyridine calcium channel antagonists.[102] Cases of myopathy, including rhabdomyolysis, have also been reported in patients with hypertension who are taking mibefradil concomitantly with statins metabolized by the CYP3A4 pathway.[103] The reason for these adverse effects is that all these drugs are first-pass compounds, with low and variable oral bioavailability. Therefore, when given concurrently with mibefradil, they may reach toxic concentrations after normal doses in some patients, largely exceeding the 'average effect' observed in the preliminary interaction studies in relatively healthy individuals.[98]

Unlike other statins, cerivastatin is metabolized by dual CYP2C and CYP3A enzymes. This and the moderate tissue distribution were considered to support a lower propensity to DIs than other statins, and appeared to confer an excellent safety profile. In combination with its increased efficacy in elderly patients and in patients with hypertension and coronary heart disease, cerivastatin was considered particularly suitable for these subgroups. Unexpectedly, however, after marketing authorization, the high incidence of myopathies and rhabdomyolysis occurring in patients receiving cerivastatin resulted in significantly greater morbidity compared with the other statins.[8,99] The risks were higher in patients using fibrates (mainly gemfibrozil) and in elderly patients, particularly women of low bodyweight using the high dose of cerivastatin.[7]

In retrospect, it can be concluded that it is important to investigate a DI in the population for whom the drug is intended, because this is the only way to assess the real effects of the disease state on such interactions.

1.2 Tools Currently in Development

1.2.1 Humanized Rodent Models

To overcome the problem of species differences in nuclear receptor activation, PXR-humanized mouse models were recently generated by cDNA transgenesis[104] or bacterial artificial chromosome transgenesis in PXR-nul mice.[105] The PXR ligands mimicked the human response to specific inducers with the CYP3A genes being strongly induced by rifampicin but not by pregnolone-16α-carbonitrile. This suggested that PXR-humanized mouse models may become useful for the prediction of potential metabolic interactions of drugs capable of activating human PXR, although confounding factors may be the presence of other mouse enzymes relevant for drug metabolism and species differences on the receptor's target gene repertoire. Although reporter gene assays have also been developed for predicting drugs that bind to PXR, there are drawbacks to their use and, at present, these assays are considered mostly for high-throughput screening for lead optimization.[75,106]

As an alternative approach to the problem of species differences in the ligand/substrate specifities of PXR/CAR receptors, Scheer et al.[107] generated and characterized a panel of new mouse models reflecting these receptors' signalling pathways. The panel contains single-humanized and knockout (KO) models of PXR and CAR and all possible combinations of these alterations, including double-humanized and KO models for both receptors. In functional studies the humanized receptor mice showed species-specific differences in interactions with known drugs: humanized PXR was strongly activated by rifampicin but only weakly by dexamethasone and pregnenolone-16α-carbonitrile; humanized CAR was potently activated by 6-(4-chlorophenyl) imidazo-[2,1-b][1,3] thiazole-5-carbaldehyde-O-(3,4-dichloro-benzyl) oxime, while 1,4-bis [2–(3,5–dichloropyridyloxy)] benzene barely activated the human receptor at all. Therefore,

this panel may offer a toolbox of mouse models to study the crosstalk of PXR and CAR *in vivo* and to evaluate their individual contributions to drug and chemical efficacy and safety in humans.

Other nuclear receptors may be involved in enzyme induction, including the aryl hydrocarbon receptor (AhR), the estrogen receptor-α[108] and glucocorticoid receptor.[109] The AhR receptor regulates the expression of genes in the CYP1 family.[110] Its ligands include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons.[111] There are large species and strain differences in sensitivity to TCDD, which are strongly influenced by functional polymorphisms of the AhR receptor.[112] Similar studies have implicated the AhR in differences in sensitivity to TCDD among avian species. The tern AhR has lower binding affinity and reduced ability to support TCDD-dependent transactivation than the chicken or mouse. These differences between avian species' AhR reside in the ligand-binding domain, and two amino acids (Val-325 and Ala-381) are responsible for the reduced activity in some species, providing a molecular understanding of species differences in sensitivity to TCDD-like compounds.[113]

Obviously, a critical question in risk assessment of TCDD-like compounds is whether humans are sensitive or resistant to their toxicity. To characterize responses mediated by the human AhR, Moriguchi et al.[114] generated a genetically modified mouse carrying the human AhR that may better predict the biological effects of bioaccumulative environmental toxicants such as TCDD in humans.

1.2.2 Cell Line-Based Assays

Among cell line-based assays, human hepatocyte cell lines (mainly originated from tumours) have indefinite proliferative capacity but generally lack a substantial set of liver-specific functions, especially major CYP-related enzyme activities, making them unrepresentative of the *in vivo* liver.[47,48] The cell line most used in CYP3A4 reporter gene assays is the human hepatocarcinoma-derived HepG2 line, although it expresses high levels of the fetal CYP3A7 instead of CYP3A4.[115] However, Aninat et al.[116] described the initial characterization of HepaRG cells which, when differentiated by dimethyl sulfoxide 2% express nuclear receptors as well as high levels of the major CYP enzymes involved in drug metabolism (CYP1A2, 2B6, 2C9, 2E1, 3A4) that are comparable to those found in cultured primary human hepatocytes. These cells also express other functions, such as phase II enzymes, apical and canalicular adenosine triphosphate-binding cassette transporters, and basolateral solute carrier transporters with albumin as well as aldolase B, which is a specific marker of adult hepatocytes.[117] The longer stability than with primary hepatocytes makes these human hepatoma cells an attractive alternative for high-throughput applications.

The non-tumourigenic immortalized human hepatocyte line Fa2N-4, described by Mills et al.[118] also demonstrated the inducibility of CYP3A4 and other PXR-regulated genes (CYP2C9, MDR1 in response to prototypical inducers, although CAR selective mechanisms and some hepatic uptake transporters appear lacking in these cells.[48,119] Using this model, a novel approach (the 'relative induction score') was developed and proposed for predicting the magnitude of clinical DIs caused by induction of CYP3A.[120]

Because CYP3A4 is present in the gastrointestinal tract and contributes significantly to presystemic drug metabolism, other groups use the human colon carcinoma-derived LS180 cell line as a host for the CYP3A4 reporter gene assay.[121,122] In comparison with other colon carcinoma cell lines (CaCo-2, TC-7), only LS180 showed inducible CYP3A4 expression.[123] Comparison of the CYP3A4 reporter activities in LS180 and HepG2 cells after PXR activation revealed that the induction of CYP3A4 reporter activity in LS180 was approximately double the induction in HepG2 for both PXR and CAR.[124]

1.2.3 In Silico Simulation Tools

As can be understood from the studies and reviews mentioned, new drug candidates that are susceptible to extensive metabolism may be involved in DIs when coadministered with substrates and inhibitors biotransformed by the same enzymes, or their metabolism may be induced by

certain xenobiotics. Predictions of the primary metabolic site from the molecular structure may, therefore, assist in identifying the metabolite formed through specific metabolic reactions and directing the metabolism to certain CYP enzymes in the effort to guide chemistry in the synthesis of new compounds with lower metabolic liability and potential for DIs. One way to predict such compounds, as anticipated in section 1.3, is by simulation, and several computational methods for predicting substrates and inhibitors of CYP enzymes, as well as the ligand of PXR, CAR and AhR, are emerging. These include ligand-based approaches (quantitative structure-activity relationships and pharmacophores), protein-based methods and homology models whose predictive power, however, needs to be improved before they can be routinely used in DI studies, as reviewed elsewhere.[125-128]

A new DI prediction framework called 'ontology-driven hypothetic assertion' (OHA), which focuses mainly on automatic generation of drug-metabolic pathways, automatic detection of DIs in multiple-drug regimens and quantitative evaluation of the DIs with numerical simulation, has also been developed. The effectiveness of the system was demonstrated in the prediction of quantitative interactions between irinotecan and ketoconazole. Pending further evaluation, the OHA method may offer a further promising approach for *in silico* prediction of *in vivo* DIs involving CYP3A4.[129]

Machine learning methods such as support vector machines, probabilistic neural network and k-nearest neighbour have been employed for predicting substrates and inhibitors of several CYP isoenzymes and transporters.[130-132] Ung et al.[133] have used these three machine learning methods for predicting PXR activators; by incorporating feature-selection methods such as recursive feature elimination, molecular descriptors relevant to PXR activators can be identified. Most of these descriptors are consistent with those used in previous pharmacophore and quantitative structure-activity relationship studies and with x-ray crystallography findings.

Finally, Yap and Chen[134] further explored the use of two consensus support vector machine methods, 'positive majority' and 'positive probability', for predicting inhibitors and substrates of CYP3A4, CYP2D6 and CYP2C9. Both methods appeared potentially useful as filters, with the 'positive probability' procedure performing slightly better than the 'positive majority' method.

2. The Translation of Knowledge from Research to Clinical Practice

There is no doubt that DIs are important in clinical practice, especially with the increasing number of elderly people[18,34,94,135] with multiple chronic diseases exposed to polytherapy[31,32,93,136] and/or with impairment of organ functions.[97,137,138] A recent study found that 2–3% of all adults in a US health maintenance organisation (HMO) were exposed in 2001 to a potential DI,[139] and a more recent review[17] found that DIs were responsible for 0.05% of emergency department visits, 0.6% of admissions and 0.1% of re-hospitalization. However, although many studies have documented the clinical relevance and economic burden of adverse events associated with DIs, the results differ widely depending on the selection of patients, age and co-morbidity, and on whether potential or actual interactions were considered.

Juurlink and colleagues[18] showed how the incidence of hospital admission for drug-related adverse events such as glibenclamide-induced hypoglycaemia was 6-fold more likely in patients given the interacting drug cotrimoxazole in the week before admission. Patients with digoxin toxicity were approximately 12-fold more likely to have received clarithromycin in the week before admission, and those treated with ACE inhibitors with a diagnosis of hyperkalaemia were approximately 20-fold more likely to have been treated with a potassium-sparing diuretic in the week before admission. An increased risk of drug toxicity was not found for drugs with the same indication but without known interactions (respectively, amoxicillin, cefuroxime and indapamide). The lack of definite information about the epidemiology and relevance of actual DIs in clinical practice can result in over- or underestimation of the clinical consequences, and leave the physician without clear

Table IV. Main risk factors for drug interactions

Patient related
Polypharmacy and inappropriate prescribing
Number of physicians prescribing drugs
Use of over-the-counter drugs, dietary supplements, food (e.g. milk, grapefruit juice), alcohol, natural remedies
Age-related pharmacokinetic and pharmacodynamic changes
Atypical presentation of disease or complaints, such as confusion, falls, urinary incontinence, weakness, etc.
Chronic or unstable diseases (diabetes mellitus, arrhythmias, epilepsy, autoimmune diseases, cancer, chronic obstructive pulmonary disease, etc.)
Organ failure (kidney, liver, heart)
Genetic variability (genetic polymorphisms)
Drug related
Narrow therapeutic index and dose-related toxicity (digoxin, hypoglycaemic agents, warfarin, antiarrhythmic drugs, phenytoin, theophylline, tricyclic antidepressants, lithium, antiepileptic drugs, etc.)
Drugs that affect vital functions (antidiabetic drugs, anticoagulants, opioid analgesics, antiarrhythmics, etc.)
Inhibitors and inducers of drug-metabolizing enzymes and transporters
Drug combinations resulting in renal dysfunction, electrolyte disorders, hypotension, bradycardia, fluid retention (renin-angiotensin system inhibitors, diuretics, NSAIDs, antihypertensives, digoxin, α-adrenoceptor antagonists, etc.)
Drug combinations with additive sedative effects (such as barbiturates, opioid analgesics, benzodiazepines, histamine H_1 receptor antagonists [antihistamines]), which may lead to falls, confusion, aspiration pneumonias, apathy and incontinence
Drug combinations with anticholinergic effects (such as antipsychotics, some antiarrhythmics [disopyramide], some tricyclic antidepressants, antihistamines, antiparkinsons, incontinence agents, etc.)
Drug combinations resulting in inadequate control of diabetes (such as antidiabetic drugs, β-adrenoceptor antagonists, atypical antipsychotics, corticosteroids, antibacterials, etc.)
Drug combinations resulting in anaemia or bleeding complications (such as NSAIDs, salicylates, selective serotonin reuptake inhibitors, anticoagulants, corticosteroids, some antibacterials, etc.)
Newly approved drugs

answers to questions such as 'what drugs are at greatest risk?', 'what mechanisms are most important?', 'how can DIs be detected?' and 'which instruments might be useful for evaluating and preventing serious ADRs?'.[34]

How can physicians evaluate and detect clinically relevant DIs starting from the preapproval information? And how can the clinical and epidemiological evidence on the determinants of DI risk help clinicians identify patients or situations at risk and avoid combining drugs potentially responsible for severe untoward effects?

Premarketing studies are often excessively oriented toward the drug and only in a few cases toward the patients because they mainly aim to identify which drugs interact with others in order to collect the information required for approval by regulatory agencies and to avoid liability.[2,3,20,35] Many of these studies evaluate 'surrogate endpoints' such as pharmacokinetic or pharmacodynamic parameters that cannot be directly translated into clinical practice or applied to the complexity of patients at risk.[26,29,140,141] Thus, it is essential to strengthen the quality of data and make it more easily translated to the individual patient.

All the information collected in premarketing studies should serve as the starting point for a more comprehensive approach at the bedside,[29,30,140,141] which should take into account all other determinants and risk factors (table IV) that largely govern the risk of DIs: patients, drugs and the contribution of DIs to adverse outcomes.

2.1 The Patient

Elderly patients are at higher risk than younger and healthy people because so many are exposed to polypharmacy or have multiple chronic comorbidities and age-related organ dysfunction that can cause pharmacokinetic changes and increase the risk of untoward effects.[18,31,93-95,142] Pharmacodynamic interactions are also proving

to be a major source of concern in this population and are sometimes unpredictable. For example, the elderly are frequently treated with low-dose aspirin (acetylsalicylic acid) and NSAIDs, but unlike other NSAIDs, ibuprofen can reduce the effectiveness of aspirin. In fact, the antiplatelet and cardioprotective effect of low-dose aspirin may even be antagonized by prior administration of ibuprofen, which is a competitive reversible inhibitor of platelet cyclo-oxygenase and interferes with the binding of aspirin to cyclo-oxygenase 1, leading to a temporary rather than sustained depression of thromboxane formation and thromboxane-dependent platelet function.[143-146]

Physicians are often not aware of all the medicines elderly patients are taking, and only rarely can they assess whether the drugs taken are appropriate or involve a risk of DIs.[94,147] Often these drugs have been prescribed for specific problems by other physicians who did not examine the drugs already used,[1,147,148] and the detection of ADRs related to DIs may be masked or confused by atypical presentation of disease or by complaints such as falls, confusion, weakness and urinary incontinence.[92]

Other populations at risk are children and women. Children are often merely considered 'small versions' of adults, instead of a population with their own physiology in whom drug action may result in or reflect important differences in metabolism and disposition, which may lead to differences in clinical response to drugs and consequently to the risk of ADRs.[149,150] Approximately 50–70% of drugs prescribed to infants and children have not been adequately studied to provide appropriate labelling information.[151] Off-label use is a measure of the lack of knowledge concerning paediatric treatments. A review of articles on off-label and unlicensed drug use in children shows that it is common and the most reported drugs are paracetamol (acetaminophen), cisapride, chloral hydrate, salbutamol and amoxicillin. A lack of harmonization exists between the evidence, information available to clinicians and its use in clinical practice, and this is partly why off-label drug use is so common.[152,153] The use of antidepressants in children is increasing, although the evidence of effectiveness and safety for depressive disorders in this population is scant and widely debated.[154]

Similarly, dosing recommendations are rarely made according to sex, although the scientific literature recognizes the existence of pharmacokinetic and pharmacodynamic differences between males and females.[85,155,156] Some ADRs are more prevalent in females, particularly elderly women.[86] For example, the toxic effects for which mibefradil was withdrawn were more frequent in elderly women.[98] Other drugs removed from the market showed greater toxicity in women than men, such as cisapride and terfenadine, whose DIs led to greater adverse pharmacodynamic consequences in a form of a ventricular tachyarrhythmia, termed torsades de pointes (TdP), associated with prolongation of the corrected QT interval on the electrocardiogram.[157] Males and females show different sensitivities to many QT-prolonging drugs and such drugs are considered a major risk factor for TdP (a list of drugs associated with TdP is available at http://www.torsades.org/).

In patients exposed to polypharmacy, all drugs prescribed should be regularly reviewed, withdrawing any identified as inappropriate or useless. Furthermore, because DIs may not be immediately obvious, patients should be closely monitored when combinations of drugs are first prescribed.[34,158]

Finally, when available, pharmacogenetic factors must be considered because in all populations different genotypes and polymorphisms may lead to different consequences from interacting drugs.[159,160] The metabolic activity of patients who lack an active enzyme (i.e. PM) cannot be increased or decreased by xenobiotics that induce or inhibit that enzyme. Notably, specific drug DIs can convert an EM to phenotypic PM status by competitively inhibiting polymorphic enzymes.[161] Again, while this may be a minor issue in the clinical trial setting where concomitant drugs can be carefully screened, it may have substantial importance in a traditional medical care setting where patients are far more difficult to monitor.

2.2 The Drug

All drugs have specific pharmacokinetic and pharmacodynamic characteristics that must be carefully considered before prescription. Although a few thousand DIs have been reported, clinically significant ones are far fewer and frequently involve drugs with a low therapeutic index, such as oral anticoagulants, some oral antidiabetic drugs, anticonvulsants, antidepressants, antiarrhythmics (including digoxin), NSAIDs (including aspirin), neuroleptics, many anticancer and immunosuppressive agents, and theophylline.[16,30,34,136] DIs are also likely to be particularly important when elimination of a drug occurs primarily through a single metabolic pathway and when a drug has non-linear pharmacokinetics or when the interaction results in conversion from linear to non-linear pharmacokinetics. The frequency of use and the doses of some drugs may also decide their importance in relation to some DIs. This is the case with NSAIDs, which are widely prescribed to patients already taking corticosteroids, anticoagulants, ACE inhibitors and other antihypertensives.

Particular caution must be exercised with drugs causing enzyme induction or inhibition. Substrates, inducers or inhibitors of drug transporters should also be carefully monitored.[162] Inhibition of the transporting function of Pgp can cause clinically significant DIs and also increase the drug accumulation in tissues such as brain and therefore increase the risk of CNS toxicity. For example, the administration of loperamide together with quinidine, an inhibitor of Pgp, induces respiratory depression because of increased entry of loperamide into the CNS.[163] Conversely, Pgp inhibition can be useful in patients who are unresponsive to conventional antiepileptic drugs. For example, verapamil, by facilitating the brain penetration of the antiepileptic drugs, helped a patient, who was unable to breathe spontaneously and who had refractory status epilepticus, to regain consciousness.[164]

A full drug history is, therefore, essential to identify all of the drugs the patient is taking and to show if the patient is 'at risk'. In this assessment the physician can be helped by computerized DI software or by therapeutic drug monitoring.[29,165]

Particular attention should also be focused on herbal supplements, which are frequently promoted as 'natural' and therefore safe and harmless. This is because their use is common in some populations and often physicians are not aware of their use. However, a number of case reports and well controlled clinical trials provide evidence of DIs between herbal preparations and synthetic drugs. There is increasing evidence, for example, that extracts of *H. perforatum* (St John's wort) can decrease the blood concentrations of many drugs through enzyme induction, including ciclosporin, tacrolimus, digoxin and warfarin. It is also associated with breakthrough bleeding and unplanned pregnancies when used concomitantly with oral contraceptives. As previously mentioned, this is because the active component of *H. perforatum*, hyperforin, is a potent agonist of PXR, which regulates the expression of proteins involved in the distribution and/or clearance of these drugs. Accordingly, induction of the Pgp substrate digoxin in healthy subjects appears to vary within *H. perforatum* preparations containing different amounts of hyperforin. Similarly, the hyperforin content of the extracts dictated the magnitude of the interactions with ciclosporin, which is a substrate of both CYP3A4 and Pgp (see Caccia[51] for a review of the above effects). Therefore, herbs should be appropriately studied in premarketing settings to provide information on the main constituents and their inhibitory and inductive potential, in order to alert consumers to potential herbal DIs.[166,167]

Today, textbooks and computer-assisted DI software are generally available and can serve as reference sources. However, all recommendations must be related to the patient and drug characteristics. Although it is impossible to indicate which of the available computerized DI databases is best, none are designed for analyses beyond single DIs and there are still many problems related to the updating, reliability, sensitivity and specificity of the alert.[165,168-171] Therefore, clinicians should be able to filter and capture alerts that refer to clinically significant DIs. Furthermore, there is wide variability on

how DIs are classified, the sources and validity of information and the criteria used to define the clinical relevance.[1,28,30,33,140,172,173]

2.3 The Contribution of DIs to Adverse Patient Outcomes

Little is known about how DIs contribute to ADRs that result in hospitalization and death; it is not known exactly how common DIs are. Studies on the frequency of potential DIs have reported rates ranging from <5% in outpatients to nearly 60% in patients living in long-term care facilities, and several studies have shown that between 2.8% and 4.4% of hospital admissions are associated with DIs.[34] However, these studies vary greatly regarding the instruments and criteria for assessment of DIs, participants and settings. Studies using administrative databases have examined the prevalence of DIs among ambulatory patients in various countries[14,93,139,174] and found that 2–3% of all adults in a US HMO were exposed to potential interactions. An Italian population-based study[16] found that the prevalence of clinically important potential DIs during 2004 was 2.1%, and the risk of DIs was higher in patients aged ≥65 years.

Although these studies all have some limitations and cannot be easily transferred to different international healthcare environments, they are extremely important because they supply information on patients, populations and drugs at higher risk of DIs starting from clinical outcome and not only from potential adverse events. In the study by Becker et al.,[17] NSAIDs and cardiovascular drugs were the treatments most often involved, and gastrointestinal bleeding, hypertension or hypotension and cardiac rhythm disturbances were the most common causes for admission or emergency visits.

3. Proposal for Improving the Clinical Applicability of Premarketing Evidence About DIs

The aims and needs of each single phase of drug development, the increasing number of patients at risk of DIs, the high prevalence of data on 'potential' rather than 'actual' DIs, and the objective difficulties of translating experimental evidence into clinical practice, indicate how far we still are from bridging the gap between premarketing evidence and clinical needs.[1,2,29,30,32,35,140,141] Several considerations may help underline the points that need to be improved and how all the available data could be used more effectively and efficiently at the bedside.

3.1 Preclinical and Clinical Studies

Considering the limitations of most procedures for quantitatively predicting DIs, during the development of a new drug closer links are needed between preclinical, experimental modelling and clinical studies, taking account of the entire body of knowledge. In designing clinical trials, attention must be paid to sample size and duration of therapy, co-morbidity and number of coadministered drugs. When premarketing data shows that specific populations might be at risk, active pharmacovigilance studies should be required and designed using clinically relevant endpoints.[34] In clinical practice, a drug with high interaction potential may pose a risk even when there are adequate warnings and evidence from well conducted premarketing studies.[7,8,98,175] After approval, the number and characteristics of exposed patients may significantly differ, so the frequency of an apparently rare ADR could dramatically increase.[26]

Reporting of suspected ADRs during active pharmacovigilance in drug- or patient-oriented studies should be encouraged, particularly for new drugs, because this is the most convenient system for detecting unexpected type B ADRs and for generation of hypotheses that can be subsequently tested with appropriate pharmacoepidemiological methods.[26,175]

There is only limited evidence so far to justify prospective pharmacogenetic testing or population-wide screening. Tests for single polymorphisms that affect pharmacokinetics may, in fact, account for only part of the variability in drug response, but the development of computerized electronic systems for drug prescription, which

provide information on pharmacogenetic risk and on dosing adjustment for patients whose genotypes are known, could be an important advance for translating pharmacogenetics into daily patient care,[176] such as in the case of warfarin dosing. A specific algorithm is available at http://www.warfarindosing.org.

Moreover, with the growth of data concerning individual differences in drug response and molecular interactions, *in silico* methods, integrating biomolecular information, pharmacogenomics, ontology and pharmacokinetics, offer promise for understanding the dynamic behaviour of drug metabolism and its potential role in DIs.[129] These methods should help in addressing the question of optimal drug combinations, evaluating variability in the occurrence of DIs, predicting the dosing and time course of DIs, and the choice of specific clinical endpoints.

Unfortunately, these prediction systems at present can evaluate only single DIs; future development should aim to test multiple drug regimens simultaneously. Further studies should be done on already known clinically relevant DIs to validate these technologies.

3.2 Accessibility of Information

Although the SPC is among the most frequently consulted information sources that clinicians use to obtain information on potential DIs and the legal basis of prescribing, it still has several limitations. Bergk et al.[28] found approximately 16% of clinically relevant DIs were missing, and information on possible outcomes and management recommendations was less precise and comprehensive than those provided by DI standard sources for >60% of the evaluated combinations. Therefore, pharmaceutical companies and regulatory agencies must make efforts to keep the SPC updated to improve its readability, comprehensibility, clinical relevance and usefulness.

At the same time, clinical scientific journals should explicitly require authors to report detailed information on DIs to make it easier for clinicians to assess the frequency, clinical relevance, outcomes, and any alterations recommended in dosing arrangements or patient management. Moreover, all information about the potential or actual risk of DIs obtained before and after the marketing of a new drug should be made readily and publicly available. Specific, independent and easily accessible drug information compendia (such as national formularies, DI electronic databases or software for personal digital assistants) should be freely accessible over the internet or integrated in the patient's electronic form and be directly consultable when clinicians prescribe drugs. Such systems should also provide specific visual or acoustic alerts for drugs with the highest risk of DIs, tables with CYP and Pgp substrates, enzyme and Pgp inducers and inhibitors, and lists of drugs that can be used as alternatives to potentially interacting drugs.

3.3 Clinicians' Education

One last but no less important element that needs to be improved is the education of clinicians: are they ready and accustomed to consult and use appropriately the available information and supporting systems? The simple approach of consulting DI lists, textbooks, leaflets or computerized databases is not always sufficient for finding answers to questions on the risk and outcome of a potential DI in a particular patient. The information provided by these instruments should serve as only the first step (the 'signal' or 'alert') in a fuller process of clinical assessment that should take into account the patient and drug characteristics, benefit-risk ratio of the new drug in light of the other medicines already taken, severity of the potential DI and how untoward effects of potential DIs could be prevented or managed when the coadministration of interacting drugs cannot be avoided.

Useful clinical algorithms are available to help clinicians assess patient risk factors, benefit-risk profiles of prescribed drugs, type, and severity and management of potential DIs.[29,140] These algorithms try to shift the attention from a drug- or disease-oriented approach to a patient-oriented approach, bringing together preclinical, premarketing and clinico-therapeutical evidence.

From the clinical perspective, physicians should be warned that in spite of the identification of different DIs, each of which may be considered

acceptable if standing alone, the same interaction can result in an unacceptable risk when the drug is prescribed to patients with multiple chronic diseases or who are receiving polytherapy.[15,17,18,141] All too often the appearance of an adverse event leads the clinician to blame the 'guilty' drug rather than making a global assessment of all the medicines the patient is taking. This mistake can induce the physician to prescribe another drug (so called 'cascade prescribing') in order to manage a misinterpreted ADR.[177]

In practice, during clinical assessment physicians must bear in mind that:

- the same disease(s) can mask or modify the clinical appearance of a DI (e.g. the hepatotoxic effects of paracetamol metabolites are increased by the presence of chronic hepatic diseases and by the coadministration of enzyme inducers);
- inter- and intraindividual variability in drug response can be due to genetic or environmental factors (as mentioned in sections 1.1.2 '*In Vitro* Human Models' and 1.1.3 'Studies in Healthy Volunteers');
- only in a few cases can the effect of a drug be easily controlled or quantitatively monitored and measured (this is the case of DIs involving, for example, antidiabetics, anticoagulants, antihypertensives and/or statins for which it is possible to measure the blood glucose, International Normalized Ratio [INR], blood pressure, serum cholesterol and creatine kinase, or in the case of DIs involving drugs such as antiepileptics, lithium, digoxin, ciclosporin and methotrexate for which therapeutic drug monitoring can be used), while drugs such as analgesics, NSAIDs, antidepressants and benzodiazepines are harder to monitor;
- the failure to recognise a DI may reflect a lack of knowledge or superficiality by the physician and/or patient because of inadequate information.

4. Conclusions

The withdrawal of some recently marketed drugs has raised public concerns on the gap between what is known at the time of approval of a new medicine and the risk of serious adverse effects due to DIs, especially in high-risk patients who are usually excluded from the premarketing phases of drug development.[4]

Clinicians, pharmacists, regulatory agencies and pharmaceutical companies must therefore recognise the need for an integrated, multidisciplinary approach in order to improve the links between experimental evidence, modelling, computer-based support systems and clinical needs.

Preclinical and premarketing study design needs to be improved to make information easily accessible and clinically transferable. Studies should include not only *in vitro* modelling, healthy volunteers and pharmacogenetic assessment of inter- and intrasubject variability in metabolic activity, but should also take into account appropriate sample size, duration, co-morbidity, number of coadministered drugs, within- and between-subject variability, specific at-risk populations and/or drugs with a relatively narrow therapeutic window, and clinical endpoints. Furthermore, after premarketing development in situations where there is potential high risk of serious adverse events, specific phase IV studies (and/or active pharmacovigilance studies) should be required to monitor and quantitatively assess their clinical impact.

The goal is to make all available DI information easily accessible and transferable in clinical practice to help clinicians address patients' needs in the best possible way.

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Correspondence: Dr *Alessandro Nobili*, Istituto di Ricerche Farmacologiche 'Mario Negri', via Giuseppe La Masa 19, 20156 Milan, Italy.
E-mail: nobili@marionegri.it