Human biology-based drug safety evaluation: scientific rationale, current status and future challenges

Abstract

Introduction: The animal toxicity studies used to assess the safety of new candidate pharmaceuticals prior to their progression into human clinical trials are unable to assess the risk of non-pharmacologically mediated idiosyncratic adverse drug reactions (ADRs), the most frequent of which are drug induced liver injury and cardiotoxicity. Idiosyncratic ADRs occur only infrequently and in certain susceptible humans, but are caused by many hundreds of different drugs and may lead to serious illness.

Areas covered: Idiosyncratic ADRs are initiated by drug-related chemical insults, which cause toxicity due to susceptibility factors that manifest only in certain patients. The chemical insults can be detected using *in vitro* assays. These enable useful discrimination between drugs that cause high vs. low levels of idiosyncratic ADR concern. Especially promising assays, which have been described recently in the peer-reviewed scientific literature, are highlighted.

Expert opinion: Effective interpretation of *in vitro* toxicity data requires integration of endpoints from multiple assays, which each address different mechanisms, and also must take account of human systemic and tissue drug exposure *in vivo*. Widespread acceptance and use of such assays has been hampered by the lack of correlation between idiosyncratic human ADR risk and toxicities observed *in vivo* in animals.

Keywords

Adverse drug reactions, liver injury, cardiotoxicity, *in vitro* assays.

Abbreviations

ADR	Adverse drug reaction
DILI	Drug induced liver injury
RM	Reactive metabolite
BSEP	Bile salt export pump
CYP	Cytochrome P450
DAMP	Damage associated molecular pattern
HLA	Histocompatibility antigen
THLE	SV40 large T antigen immortalised human liver epithelial cell line
HERG	Human ether-à-go-go related channel
$C_{max,ss}$	Maximum steady state drug concentration
IC_{50}	Compound concentration causing 50% inhibition of observed effect
EC_{50}	Compound concentration causing 50% of maximal observed enhanced effect
LTKB	Liver Toxicity Knowledgebase
MRP2	Multidrug resistance-associated protein 2
PBPK	Physiologically based pharmacokinetic
RM	Reactive metabolite

1. Introduction

Before new pharmaceuticals progress into clinical trials they undergo extensive safety testing in animals. These studies are mandated by regulatory guidelines [e.g. 1,2]. Their purpose is to reduce, and ideally eliminate, the likelihood that hazardous effects will arise in humans. They include evaluation in experimental animals of acute (single dose) and repeat dose toxicities, skin sensitisation, carcinogenicity, reproductive toxicity, neurotoxicity, developmental toxicity and embryotoxicity [3,4]. However, it is clear that the use of animal safety studies to assess drug safety has numerous important limitations.

Animal studies require substantial amounts of test compound, are relatively expensive and can only be undertaken on relatively few compounds. In addition, they deliver results quite slowly, over the course of many weeks or months. Therefore they cannot be used to design and select safe drugs during early phases of drug discovery, when there is abundant chemical choice but only small amounts of test compounds are available and data must be provided rapidly in order to align with the short timeframe (generally 2 weeks) of the routine design/make/test assess cycle. This is why many potential candidate drugs exhibit toxicities in animal safety studies which prevent their progression into clinical trials [5,6].

Furthermore, animal safety studies have only limited value for prediction of human adverse drug reactions (ADRs). When 12 multinational pharmaceutical companies undertook a retrospective survey of 150 small molecule drugs that had progressed into clinical trials, they discovered an overall concordance of 70% between toxicities observed in animal safety studies and human ADRs [3,7]. The highest animal / human concordances (>80%) were seen for haematological, gastrointestinal and cardiovascular ADRs, whereas markedly poorer concordances (<55%) were observed for liver and skin toxicities [3,7]. The animal studies had been performed in rodents (primarily the rat) and non-rodents (primarily the dog). Even

lower human/animal concordances were evident when safety studies undertaken in rodents alone (43%) or non-rodents alone (63%) were considered.

Some ADRs are due to exaggerated drug pharmacology. These include bleeding caused by anticoagulants, stomach ulceration caused by nonsteroidal anti-inflammatory drugs and hypotension caused by anti-hypertensives. Typically, such ADRs occur relatively frequently, exhibit clear dose dependence and often can be anticipated before clinical trials are undertaken. However, many hundreds of licensed drugs cause serious ADRs that are unrelated to drug pharmacology and occur only infrequently (typical incidence <1:1000), in certain susceptible individuals. These rare toxicities arise due to a combination of adverse biological effects elicited by the drugs plus unusual biological responses that arise only in susceptible individuals, hence are termed "idiosyncratic" ADRs. They may cause severe and potentially life threatening organ toxicities, of which the most frequent is drug induced liver injury (DILI), immune mediated hypersensitivities, haematologic and skin reactions [8,9,10].

ADRs that occur during human clinical trials are an important cause of terminated development of new candidate drugs [10,11]. Project failures due to animal toxicities or human ADRs are leading reasons why drug discovery and development is a highly inefficient and very costly process [5,6,9,10]. Clinical development of drugs that cause ADRs may continue if the expected clinical benefit arising from use of a new drug is considered sufficiently important to outweigh the anticipated ADR risk. This helps explain why many licensed drugs cause clinically important ADRs, which result in cautionary and restrictive labelling that is intended to inform and help protect patients [12,13]; and why ADRs are a leading cause of serious human ill health requiring hospitalisation in the USA, UK and other countries [14,15,16]. Idiosyncratic ADRs pose a particular problem because typically they are not observed in animal safety studies and are not recognised until very late in clinical development or even following registration. Some selected examples are listed in **Table 1**

[17]. When the clinical consequences are sufficiently concerning they may lead to withdrawal of previously licensed drugs from clinical use. Examples of drugs withdrawn since 2000 due to idiosyncratic ADRs are listed in **Table 2** [18-34].

In view of the many limitations of drug safety studies in animals, it is imperative to develop alternative approaches that are more predictive of human ADRs and can be used to design and select safe new drugs. *In vitro* assays that utilise human tissue-derived cells or organelles are a very promising alternative. The scientific rationale for their use is discussed in section 2. Some especially promising assays are highlighted in section 3, as are case examples that illustrate their potential value. The scientific and other challenges that must be surmounted to enable more widespread acceptance and use of *in vitro* assays are discussed in section 5.

2. *In vitro* assay scientific rationale

ADRs are initiated by drug induced chemical insults to the relevant target cells and tissues. Toxicologically relevant insults can arise in many different ways. These include off-target secondary pharmacological interactions [35], mitochondrial injury [36], damage to lysosomes, peroxisomes and other organelles [37], initiation of programmed cell death (apoptosis or necroptosis) [38,39], plasma membrane transporter inhibition [40] and activation of deleterious immune responses [41,42]. They may be caused by the drugs themselves or by their metabolites, most notably unstable and chemically reactive metabolites (RMs) that react non-covalently with oxygen or lipids, or covalently with DNA or proteins [43]. Cells have developed a variety of mechanisms which protect them from injury that may arise via these mechanisms. These include the glutathione system (which protects against cellular injury caused by electrophilic RMs) [44], various other stress response processes (which include heat shock protein and glucose regulated protein systems, the antioxidant

response and the nrf-2 transcriptional cascade, and endoplasmic reticulum stress responses), cytoprotection mediated by activation of the innate immune system and tissue repair processes [41,45].

The importance of these processes in protection against ADRs is illustrated by the widely used analgesic paracetamol. When taken at recommended therapeutic doses (up to 4g/day in adults), paracetamol is metabolised primarily to non-toxic metabolites and although small amounts of RM are formed in the liver, DILI does not occur because these are detoxified by reactivity with glutathione. However, when high doses of acetaminophen are ingested, large amounts of RM are formed. These overwhelm the protective capacity of the glutathione system and initiate a complex cascade of intracellular and intercellular events (glutathione depletion, oxidative stress, covalent modification of hepatocyte proteins, cytokine release, inflammatory cell infiltration and activation of the innate immune system), which results in necrosis and release of damage-associated molecular patterns (DAMPs) [46,47]. As a consequence of DAMP release, the innate immune system is activated causing cytokine formation and inflammatory cell infiltration, which are necessary to remove necrotic cells but also have the potential to aggravate the original liver injury [48].

Since idiosyncratic ADRs occur only in susceptible humans, and cannot be reproduced in the animal species used in preclinical safety assessment of new pharmaceuticals, the mechanisms by which they occur are less clearly defined. Important insights have been obtained by investigators who have undertaken studies in strains of animals which differ from those used in standard regulatory drug safety studies, and/or following pre-treatment of animals to enhance their susceptibility to toxicity [49]. For example, autoimmune reactions have been demonstrated in Brown Norway rats dosed with penicillamine [49], while skin rash was observed in female Sprague-Dawley or Brown Norway rats dosed with nevirapine [50]. In addition, liver injury was produced when rats were pre-treated with lipopolysacharide to

stimulate inflammatory stress, then were exposed to various drugs found to cause human idiosyncratic human DILI [51]. Furthermore, liver injury was evident when strains of mice with impaired immune tolerance (Cbl-b(-/-) or PD-1(-/-)) were co-administered anti-CTLA-4, to deplete regulatory T cells, and amodiaquine (which in humans causes rare idiosyncratic DILI) [52]. Similarly, liver injury was produced when female Balb/cJ mice were treated with anti-Gr-1 antibody, to deplete suppressor T cells, then dosed with halothane [53]. These and other non-standard animal studies have provided valuable insights into the complex mechanisms by which idiosyncratic ADRs may arise, and in particular have highlighted the key contributions of innate and adaptive immune reactions. However, such studies are highly resource intensive and are unable to deliver data that aligns with the short turnaround times required during compound optimisation in drug discovery. In addition, currently it is unclear whether any single animal model (or small selection of animal models) can proactively detect all clinically concerning idiosyncratic ADRs with high sensitivity and specificity. Hence these models are not used routinely to support non-clinical safety assessment of new pharmaceuticals."

Many drugs which cause idiosyncratic ADRs are metabolised to RMs [54,55] and/or can initiate toxicity in other ways; these include mitochondrial injury [36], cell cytotoxicity [56,57], inhibition of the liver-specific Bile Salt Export Pump (BSEP) [58,59] and interactions with ion channels on cardiac cells that regulate cardiac contractility [35]. In addition, drug-specific antibody mediated and/or cellular immune responses patients have been demonstrated in patients who develop idiosyncratic ADRs caused by numerous drugs [60,61], while associations between individual ADR susceptibility and HLA haplotype have been demonstrated for a number of drugs [62]. In view of these findings, plus data obtained from the *in vivo* investigative studies undertaken in animals and discussed in the previous paragraph [49-53], it can be inferred that idiosyncratic ADRs arise due to a combination of

compound-related initiating mechanisms and patient-related susceptibility factors that which manifest only in susceptible individuals and include drug-induced innate adaptive immune responses [63,64]. This is illustrated schematically in **Figure 1**, which also highlights that the early drug-related events which initiate these ADRs can be investigated using *in vitro* assays.

3. Use of *in vitro* assays to identify drugs that cause human ADRs

An ideal *in vitro* toxicity screening strategy needs to be able to assess the many different compound-related mechanisms that can initiate human ADRs, to provide highly reproducible data at reasonable cost, and also to be applicable during early phases of drug discovery when there is abundant chemical choice (i.e. provide data relatively quickly, on potentially large numbers of text compounds). In addition, the data obtained needs to have high ADR specificity and sensitivity. These are major challenges and as yet there is no overall consensus within the scientific community on how best to meet them. Nonetheless, substantial progress has been made in the last 10 years and many useful assays have been described. Due to the high level of concern raised by DILI and cardiotoxicity (see Tables 1 and 2), the primary focus of attention of this review is on assays which quantify key processes that may initiate these toxicities. Some especially promising approaches, all of which focus upon endpoints that are considered directly relevant to human ADRs, are summarised in **Table 3**. Assays which address other target organs (skin, haematopoetic etc.) are not discussed, even though these represent important idiosyncratic ADRs (see Table 1).

Some investigators have used cultured cells isolated directly from human tissues. In principle, this is especially advantageous for DILI since isolated human hepatocytes express physiologically relevant drug biotransformation enzymes and drug transporters, which are expressed only at much lower levels (or are absent) in human liver-derived cell lines. Hence

many studies have used isolated human hepatocytes (e.g. [65,67]), while others have studied hepatocytes from animal species (e.g. [71,72]). However, availability of freshly isolated human hepatocytes is limited, the cells are expensive and they can exhibit marked inter-individual variability in drug biotransformation capability. Therefore many investigators have chosen to evaluate liver-derived cell lines (e.g. HepG2 and THLE [56,57,67]), while others have investigated liver cell lines transfected with individual human drug metabolising enzymes. In particular, a panel of immortalized human liver-derived THLE cell lines has been described that were transfected with a variety of individual human cytochrome P450 (CYP) enzymes and stably expressed enzyme activities that were similar to those present in freshly isolated human hepatocytes [67]. Dambach et al. described use a panel of 5 THLE cell lines (which expressed no CYP enzymes, CYP2C9, CYP2D6 or CYP3A4) to discriminate between metabolism-independent and P450 metabolism-mediated cell toxicity [67]. Toxicity data were obtained with 697 marketed pharmaceuticals and an *in vitro* cytotoxicity potency threshold of 50 μM was used to discriminate between "toxic" and "no-toxic" compounds. This approach was reported to discriminate between 587 non-hepatotoxic drugs and 92 hepatotoxic drugs with reasonable sensitivity (72%) and high specificity (99%). In view of these findings, toxicity studies undertaken using these cell lines were introduced by one pharmaceutical company as a first tier screen, to aid early identification of potentially hepatotoxic compounds during drug discovery [67]. Data obtained subsequently by other investigators have further verified the value of *in vitro* toxicity data obtained using non-P450 expressing and P450 expressing THLE cell lines for prediction of human DILI *in vivo* [57].

Relatively few direct comparisons between data provided by human liver-derived cell lines and primary hepatocytes have been reported. Such comparisons are important because the cell lines used most frequently in toxicity studies (e.g. HepG2) exhibit only low activities of

CYP enzymes and other important Phase 1 and Phase 2 drug metabolizing enzymes, and also do not express BSEP and many of the other polarized cell surface transporters expressed on hepatocytes *in vivo*. Furthermore, HepG2 cells were derived from a liver hepatoma and so may exhibit different signalling pathways and responses to cell stress, when compared with non-transformed liver cells. The data available currently suggest that toxicity data provided by both human liver-derived cell lines and primary hepatocytes exhibit high specificity for human DILI (>80%), and that use of human hepatocytes may provide only modest improvement in human DILI sensitivity, even when multi-parametric evaluation of drug induced loss of cell viability is undertaken (e.g. [65]). Nonetheless, this is an important aspect which merits further investigation.

Most studies have evaluated single cell types cultured in static monolayer configuration [57,65-72], although more complex and physiologically relevant models have also been described. These include isolated hepatocytes co-cultured with non-parenchymal liver cells or other accessory cells [73], plus hepatocytes or HepG2 cells exposed to drugs plus pro-inflammatory cytokines [78]. In addition, multiple liver cell types have been cultured in devices that reproduce three dimensional cell-cell interactions and shear stress [74]. It can be expected that such improved cell culture conditions may enable improved *in vitro* human DILI prediction, and further enhance our understanding of the key underlying mechanisms [79].

Membrane vesicles isolated from insect cells transfected with cDNA encoding human BSEP enable quantification of human BSEP inhibition by drugs [58,59] and are especially useful for high volume compound screening. However, these assays do not provide insight into possible interactions between drugs and multiple hepatocyte transporters, which can be explored in isolated hepatocytes [71,72].

Cell lines transfected with HERG or other human cardiac ion channels have been used to study drug-induced cardiotoxicity [75], as have cardiomyocytes which were differentiated from human induced pluripotent stem cells [75,76]. All of the methods summarised in Table 3 can be used as high volume routine assays, apart from human hepatocyte covalent binding studies, which require availability of radiolabelled drugs [70] and three dimensional microfluidic devices [74,79].

When attempting to interpret *in vitro* assay data, numerous investigators have taken account of *in vivo* human drug exposure in addition to potencies of *in vitro* assay results (typically expressed as EC_{50} or IC_{50}) and have found that this markedly improved human ADR specificity. Ideally, the concentration of drug and/or toxic drug metabolites within the relevant target tissue(s) should be used when interpreting *in vitro* assay data, and account should also be taken of inter-individual differences in tissue exposure. Unfortunately, such data usually are not available and hence maximum average steady state drug concentrations in plasma or serum ($C_{max,ss}$) have been used in their place.

When assay data were adjusted to take account of *in vivo* human serum or plasma drug exposure (e.g. by calculating EC_{50} / $C_{max,ss}$ ratios), the *in vitro* methods summarised in Table 3 have each exhibited good apparent human ADR specificity (>80%) but none have exhibited high sensitivity (typically <60%). The modest sensitivity of individual assays is unsurprising, since human ADRs can occur in many different ways and all of these will not be reproduced in any relatively simple *in vitro* model. Also, most of the *in vitro* methods exhibit no or very limited drug metabolism capacity, so can be expected to underestimate markedly ADRs caused by metabolites.

Improved ADR sensitivity has been obtained by combining data provided by multiple assays. In particular, excellent discrimination between drugs with high vs. low human idiosyncratic ADR concern (which included troglitazone vs. rosiglitazone, sitaxentan vs. ambrisentan and

clozapine vs. olanzapine; see **Table 4**) was achieved when data provided by multiple *in vitro* assays were integrated and also adjusted to take account of *in vivo* drug exposure [70,90]. The assays addressed biotransformation independent THLE cell cytotoxicity, CYP3A4 potentiated THLE cell toxicity, BSEP inhibition, MRP2 inhibition and formation of chemically reactive metabolites [70,90]. Many drugs with high ADR concern exhibited multiple in vitro liabilities (e.g. troglitazone, sitaxentan, clozapine) [70,90]. However, it is important to note that the number of drugs which has been evaluated in this way is relatively small (39 in total). In the future it will be important to extend the evaluation to many more drugs, so that the value and limitations of the approach can be characterised more rigorously.

4. Conclusion

The animal toxicity studies used to assess the safety of new candidate pharmaceuticals prior to their progression into human clinical trials are unable to assess the risk of non-pharmacologically mediated idiosyncratic ADRs. Although idiosyncratic ADRs occur only infrequently and in certain susceptible humans, they are caused by many hundreds of different drugs and may lead to serious illness. Hence it is highly desirable to develop alternative approaches which enable them to be predicted and avoided.

In contrast, impressive progress has been made in the development of useful *in vitro* assays which assess mechanisms that can initiate idiosyncratic ADRs. These do not address individual susceptibility factors. Therefore they are unsuitable for use in assessing the relative ADR risk posed to individual patients. When the data provided by the *in vitro* assays were adjusted to take account of human serum or plasma *in vivo* drug exposure, they were able to distinguish with high specificity between drugs with high and low levels of idiosyncratic ADR concern in the human population. The data provided by individual *in*

vitro assays exhibited modest ADR sensitivity when analysed in isolation. The data available currently, which is based upon evaluation of a modest number of test compounds (39 drugs), indicates that high apparent ADR sensitivity may be achieved by integrating data provided by multiple assays.

5. Expert opinion

The *in vitro* approaches discussed in this article provide markedly better identification of drugs which may cause clinically concerning human idiosyncratic ADRs than the animal toxicity studies that are mandated by current regulatory guidances [1,2]. However, these *in vitro* methods have not yet achieved widespread acceptance and use by the scientific community, or by regulatory agencies.

An important reason for this is that the *in vitro* assays are intended to identify compounds which cause toxicities that cannot be detected in experimental animals, and arise only infrequently in the human population. Hence their performance must be evaluated not by comparison with animal toxicity data, but rather by comparison with ADRs which occur only infrequently in humans. Furthermore, the *in vitro* assays that are available currently address drug-related early events which can initiate ADRs, but not the susceptibility factors which determine whether they manifest in patients. They are intended to aid the design and selection of compounds with low propensity to cause human idiosyncratic ADRs, not to provide reliable data on the level of ADR risk in individual humans.

Widespread acceptance and use of these *in vitro* assays will require a major paradigm shift within the scientific community and regulatory agencies. Given the fact that currently mandated animal toxicity tests cannot predict idiosyncratic ADRs, and the magnitude of the human ill health and death that they cause, the urgency could not be greater. To aid scientific

acceptance of the assays, it will be important to explore why the mechanisms that they evaluate cause toxicities in susceptible patients, but not in non-susceptible individuals. In principle, this might be tackled by use of mechanistically relevant biomarkers that assess functionally relevant events in humans *in vivo*. Suitable examples could include perturbed bile formation caused by compounds which inhibit BSEP [90], or adaptive immune responses triggered by reactive metabolite formation [42,54,60].

Another important consideration is that the *in vitro* assays have been described only relatively recently and have been used to date by relatively few scientists. Hence their value has not yet been sufficiently widely appreciated. Furthermore, different investigators typically have developed and evaluated different *in vitro* assays and often have tested different numbers and varieties of "positive" and "negative" test compounds (i.e. drugs with high and low levels of human ADR concern), which have been selected and annotated in diverse and often inconsistent ways.

An objective and evidence-based comparison of the ADR specificities and sensitivities of the various assay options is now needed, so that the most useful approaches can be selected for formal validation. Such a comparison is a high priority, but is not possible from the currently available data. It should be based on analysis of data obtained following evaluation of a commonly agreed set of well-annotated test compounds. Promising progress has been made in DILI annotation of licensed drugs, via creation of the Liver Toxicity Knowledgebase (LTKB) [92]. The LTKB has categorised drugs based on the types and severities of DILI that they can cause, using information that is summarised in USA FDA labels, and is a notable achievement. However, many drugs cause idiosyncratic ADRs other than DILI, or in addition to DILI, (see **Table 4** [84-93]) and these are not addressed by LTKB.

Development of a suitable standard list of test compounds, whose levels of idiosyncratic ADR concern have been characterised according to an agreed set of consensus criteria that

considers multiple target organs and so can be used to evaluate the overall ADR-predictive value of different *in vitro* assays, is a key unresolved issue which needs to be tackled as a high priority.

In the future it will be important also to develop and utilise approaches that enable accurate prediction and quantification of tissue exposure to drugs and their metabolites. These are required in place of serum or plasma exposure values, to aid *in vitro* data interpretation. Furthermore, since numerous drugs which cause human ADRs exhibit multiple *in vitro* liabilities (e.g. [70,90]), the need to develop improved ways to integrate data provided by multiple assays when evaluating *in vivo* ADR risk presents an additional challenge. Use of physiologically based pharmacokinetic scaling (PBPK) methods have already provided useful simulations of human population variability in drug exposure, which have aided interpretation of *in vitro* DILI assay data [93,94]. In principle, PBPK-based approaches may be expected also to aid prediction of *in vivo* tissue exposure, plus integration of data provided by multiple *in vitro* assay methods.

Article highlights box

- Animal toxicity studies do not enable prediction of human idiosyncratic ADR risk posed by licensed drugs.
- Human idiosyncratic ADRs are initiated by chemical insults, which can be assessed using *in vitro* assays.
- Hence *in vitro* methods which address these initiating mechanisms can be used to identify drugs with high propensity to cause ADRs in the human population.
- Effective interpretation of *in vitro* assay data must take account of *in vivo* human drug exposure and requires integration of data from multiple assays.
- However, *in vitro* assays cannot be used to assess ADR risk in individual human patients.

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Tables

Table 1. Examples of licensed drugs which cause idiosyncratic ADRs.

Data have been extracted from information summarised in US FDA drug labels, which was accessed via the DailyMed Website [17]**.** This table is not a comprehensive summary of idiosyncratic ADRs. Data on the apparent frequency of the ADRs can be obtained from [17].

Drug name	Drug class	Idiosyncratic ADR
Abacavir	Antiretroviral (HIV-1 infection)	Hypersensitivity, lactic acidosis, severe hepatomegaly
Amiodarone	Antiarrhythmic	Pulmonary, DILI, thyroid, optic
Carbamazepine	Antiepileptic	Cutaneous, haematologic, agranulocytosis, cardiovascular, DILI
Amoxicillin / clavulanate	Antibacterial	Hypersensitivity, DILI, gastrointestinal, haematological
Clopidogrel	Anticoagulant (platelet aggregation inhibitor)	Haematologic, DILI, hypersensitivity
Diclofenac	Nonsteroidal antiinflammatory	Cardiovascular thrombotic, DILI, renal, cutaneous, haematological
Flutamide	Anticancer	DILI
Isoniazid	Antitubercular	DILI, haematologic, hypersensitivity
Lapatinib	Anticancer	DILI, cutaneous, cardiovascular
Nefazodone	Antidepressant	DILI, cardiovascular, cutaneous, haematologic
Phenytoin	Antiepileptic	Cutaneous, hypersensitivity, DILI, haematopoetic

Tamoxifen	Anticancer	Thromoboembolic, DILI, ocular
Ticlopidine	Anticoagulant (platelet aggregation inhibitor)	Haematologic, DILI

Table 2. Drugs withdrawn due to idiosyncratic ADRs since 2000.

Drug name	Drug use	Year withdrawn	ADR responsible for withdrawal	References
Cisapride	Gastroesophageal reflux disease	2000 (USA)	Cardiovascular	[18]
Phenylpropanolamine	Nasal decongestant, weight control	2000 (USA)	Stroke	[19]
Alatrofloxacin	Antibiotic	2001 (EU, USA)	DILI	[20]
Trovafloxacin	Antibiotic	2001 (EU, USA)	DILI	[20]
Cerivastatin	Statin	2001 (EU, USA)	Rhabdomyolysis	[21]
Levacetylmethadol	Opioid dependence	2001 (EU)	Cardiovascular	[22]
Nefazodone	Antidepressant	2003 (EU)	DILI	[23]
Rofecoxib	Nonsteroidal antiinflammatory	2004 (USA)	Cardiovascular	[24]
Pemoline	CNS stimulant	2005 (USA)	DILI	[25]
Valdecoxib	Nonsteroidal antiinflammatory	2005 (USA)	Cardiovascular, cutaneous	[26]
Ximelagatran	Anticoagulant	2006 (EU)	DILI	[27]
Lumiracoxib	Nonsteroidal antiinflammatory	2007 (EU)	DILI	[28]
Pergolide	Dopamine receptor agonist	2007 (USA)	Heart valve damage	[29]
Tegaserod	GI motility stimulant	2007 (USA)	Cardiovascular	[30]

Rimonabant	Anti-obesity	2008 (EU)	Severe depression and suicide	[31]
Sibutramine	Anti-obesity	2010 (USA, EU)	Cardiovascular	[32]
Propoxyphene	Opioid analgesic	2010 (USA)	Cardiovascular	[33]
Tetrazepam	Benzodiazepine anxiolytic	2013 (EU)	Cutaneous	[34]

Table 3. Examples of *in vitro* toxicity assays

Cell model	Endpoints assessed	References
Human HepG2 cells	High content screening of cell viability	[56,65]
Human HepG2 cells	Mitochondrial injury	[66]
Human liver THLE cell lines expressing human P450s	Cell viability	[57,67]
Rat and human primary hepatocytes	High content screening of cell viability	[65,67,68]
	Covalent binding of radiolabelled compound to proteins	[70]
	Biliary efflux inhibition	[71,72]
Membrane vesicle expressing Bile Salt Export Pump (BSEP)	BSEP activity inhibition	[58,59]
Micropatterned hepatocyte / accessory cell co-cultures	Cell viability and function	[73]
Human liver cell 3D microfluidic liver model	Cell toxicity (multi-parametric)	[74]
Human HEK293 cells transfected with hERG ion channel	Electrophysiology (QT interval prolongation)	[75]
Induced pluripotent stem cell derived human cardiomyocytes	High content screening of cell viability	[76]
	Electrophysiology (QT interval prolongation)	[77]

Table 4. Examples of pharmacologically similar drugs with markedly different idiosyncratic ADR propensities.

Drug name	Clinical use (pharmacological target)	ADR concern category	Most concerning idiosyncratic ADRs	References
Troglitazone	Antidiabetic (PPAR-γ antagonist)	Severe (withdrawn due to DILI)	Liver failure, DILI.	[80]
Rosiglitazone	Antidiabetic (PPAR-γ antagonist)	High (cardiovascular toxicity)	Black Box warning for congestive heart failure, myocardial ischaemia.	[81]
Sitaxentan	Pulmonary hypertension (endothelin receptor antagonist)	Severe (withdrawn due to DILI)	Liver failure, DILI.	[82]
Ambrisentan	Pulmonary hypertension (endothelin receptor antagonist)	Low		[83]
Clozapine	Schizophrenia (neuroleptic)	High (neutropenia, cardiovascular, seizures)	Black box warning for neutropenia, cardiovascular, seizures. Also DILI, haematologic.	[84]

Olanzapine	Schizophrenia, bipolar disorder (neuroleptic)	Moderate (cardiovascular)	Bloack Box warning for increased mortality in elderly patients with dementia -related psychosis.	[85]
Tolcapone	Parkinson's disease (catechol-O-methyltransferase inhibition)	High (DILI)	Black Box warning for DILI, cardiovascular.	[86]
Entacapone	Parkinson's disease (catechol-O-methyltransferase inhibition)	Low		[87]
Fialuridine	Hepatitis B viral infection (anti-retroviral nucleoside analogue)	Severe (withdrawn due to multi-organ failure)	Liver failure, lactic acidosis, liver and multi-organ failure.	[88]
Tenofovir	HIV-1 and Hepatitis B viral infection (anti-retroviral nucleoside analogue)	High (lactic acidosis, severe hepatomegaly)	Black Box warning for lactic acidosis and severe hepatomegaly.	[89]

Figure 1. Overview of mechanisms by which idiosyncratic ADRs arise.