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Current Challenges and Controversies in Drug-Induced Liver Injury

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Abstract

Current key challenges and controversies encountered in the identification of potentially hepatotoxic drugs and the assessment of drug-induced liver injury (DILI) are covered in this article.

There is substantial debate over the classification of DILI itself, including the definition and validity of terms such as 'intrinsic' and 'idiosyncratic'. So-called idiosyncratic DILI is typically rare and requires one or more susceptibility factors in individuals. Consequently, it has been difficult to reproduce in animal models, which has limited the understanding of its underlying mechanisms despite numerous hypotheses. Advances in predictive models would also help to enable preclinical elimination of drug candidates and development of novel biomarkers.

A small number of liver laboratory tests have been routinely used to help identify DILI, but their interpretation can be limited and confounded by multiple factors. Improved preclinical and clinical biomarkers are therefore needed to accurately detect early signals of liver injury, distinguish drug hepatotoxicity from other forms of liver injury, and differentiate mild from clinically important liver injury. A range of potentially useful biomarkers are emerging, although so far most have only been used preclinically, with only a few validated and used in the clinic for specific circumstances. Advances in the development of genomic biomarkers will improve the prediction and detection of hepatic injury in future.

Establishing a definitive clinical diagnosis of DILI can be difficult, since it is based on circumstantial evidence by excluding other aetiologies and, when possible, identifying a drug-specific signature. DILI signals based on standard liver test abnormalities may be affected by underlying diseases such as hepatitis B and C, HIV and cancer, as well as the concomitant use of hepatotoxic drugs to treat some of these conditions. Therefore, a modified approach to

DILI assessment is justified in these special populations and a suggested framework is presented that takes into account underlying disease when evaluating DILI signals in individuals.

Detection of idiosyncratic DILI should, in some respects, be easier in the postmarketing setting compared with the clinical development programme, since there is a much larger and more varied patient population exposure over longer timeframes. However, postmarketing safety surveillance is currently limited by the quantity and quality of information available to make an accurate diagnosis, the lack of a control group and the rarity of cases. The pooling of multiple healthcare databases, which could potentially contain different types of patient data, is advised to address some of these deficiencies.

Drug-induced liver injury (DILI) is the most frequent cause of acute liver failure (ALF) and transplantation in Western countries.[1] Hepatotoxicity associated with most drugs is considered idiosyncratic,[2] and typically develops in only a small proportion of subjects exposed to a drug in therapeutic doses.[3,4] The risk of ALF associated with idiosyncratic hepatotoxicants is usually less than 1 in 10 000 exposed patients.[5-7]

However, almost half of the cases reported by doctors as being caused by drug hepatotoxicity are unrelated to the suspected drug, and the lack of a reliable and widely applicable method to assess DILI may result in a severely under- or overestimated incidence.[8] More than 1000 drugs and herbal products have been associated with idiosyncratic hepatotoxicity, with the most common in clinical practice being antibacterial agents, NSAIDs and anticonvulsants.[1,9] Often, a drug's hepatotoxic potential can only be recognized postmarketing. Consequently, DILI has been the most frequent single reason for withdrawing drugs from the market or for severe reduction of use and modification of drug labelling, e.g. isoniazid, nimesulide and tolcapone.[1,10,11]

An international DILI Expert Working Group has recently published their recommendations on uniform criteria to define, characterize, diagnose and classify the full spectrum of clinical syndromes that constitute drug-induced hepatotoxicity.[12]

This article discusses challenges that are encountered by clinicians, researchers and the pharmaceutical industry in the prediction, assessment and risk mitigation of idiosyncratic DILI.

1. Definition of Drug-Induced Liver Injury (DILI)

DILI is often subdivided into 'intrinsic' and 'idiosyncratic'; however, the value and validity of this classification remains controversial. There is no conclusive evidence that intrinsic and idiosyncratic DILI occur through different mechanisms.[13] A given DILI may fall within a spectrum ranging from clear intrinsic hepatotoxicity, to that determined by individual susceptibilities.[14]

Intrinsic drug reactions result from drug- or metabolite-induced direct hepatocyte damage. They occur with short latency (within a few days) and affect the majority of the population at a large enough dose. These reactions are usually not associated with hypersensitivity manifestations, so rechallenge does not lead to earlier recurrence. Drugs that cause intrinsic DILI are usually detected in preclinical testing during development, prompting early termination with limited clinical impact.

Idiosyncratic drug reactions only occur in a minority of susceptible individuals. The reactions have variable and prolonged latency (several weeks to 1 year) and are generally unexpected on the basis of the pharmacological action of the drug. A common misconception of idiosyncratic DILI is that it is not related to drug dose.[2,15] In fact, recent data suggest that drugs given in daily doses of >50 mg and undergoing extensive hepatic metabolism appear to have a greater risk of inducing idiosyncratic DILI than lower doses.[16,17]

The infrequent occurrence of idiosyncratic DILI and its dependence on individual sensitivity factors make it difficult to detect these reactions during current preclinical testing or clinical trials. Therefore, idiosyncratic DILI presents a major issue for pharmaceutical development as well as for patient care. In order to effectively deal with idiosyncratic DILI, it is imperative to better understand the underlying mechanisms and to identify individual genetic and/or environmental susceptibility determinants.

2. Limitations of Current Models for DILI

Considering the large number of drugs associated with idiosyncratic DILI in humans, there are currently relatively few animal models in which these drugs cause overt liver injury. There are even fewer that mimic the pronounced degree of injury induced by some drugs in humans. In some models, liver injury only occurs in a small minority of treated animals and requires prolonged drug exposure, whilst others suffer from a requirement for very stringent exposure conditions to obtain liver injury.

These characteristics are consistent with human idiosyncrasy, but increase markedly the numbers of animals and amounts of drug needed for testing. Other problems encountered include species differences in genetic make-up and in the metabolism of drugs, variability in animal responses among experiments, underlying assumptions that the mode of action in the animal model is similar to human DILI, and the use of animals that are impractical for widespread application.

Better predictive models for idiosyncratic DILI would enable the preclinical elimination of drug candidates with idiosyncrasy liability, through the development of mechanism-based, *in vitro* and high-throughput tests. Any predictive model (*in silico*, cell-based or *in vivo*) might be useful, but animal models are more likely to represent the complexity of idiosyncratic reactions. In addition, reproducing these reactions in an animal model could provide evidence for a mode of action and thereby guide the identification of risk factors, improve clinical diagnosis and result in the identification of new biomarkers.

Despite the issues in developing and utilizing animal models of idiosyncratic DILI, there has been some progress (see table I) and further models should emerge in the near future.[49] Empirical *in vivo* or *in vitro* models in which endpoints correlate with human DILI responses could provide improvements in prediction compared with what is currently available. Although human mechanisms are not yet fully understood, there are several known mechanisms or proposed hypotheses for idiosyncratic DILI.

2.1 Metabolic Activation

Metabolic activation is a known mechanism of DILI. Although the use of *in vitro* tools, such as human liver microsomes, human hepatocytes, liver slices and recombinant enzymes aid in the assessment of human drug metabolism, these alone cannot predict and replicate the variable processes of pharmacokinetic and toxicologic variations *in vivo*.

A confounding factor in the development of reproducible animal models is the marked species differences between humans and other animals in response to, and in the metabolism of, xenobiotics. This is seen in the expression and activity of a number of drug-metabolizing enzymes.[50-53] The inter-individual differences of these enzyme activities in humans are often greater than differences between humans and laboratory animals.[54] In addition, sex-related variations in drug metabolism in animals may make it harder to interpret findings.[55]

As a result of these difficulties, there has been limited success in the use of murine animal models, based on genetic deletions in specific isoforms of cytochrome P450 (CYP) drug-metabolizing enzymes, in chemical toxicity and carcinogenicity studies.[56] However, CYP2E1 knockout mice have been shown to be markedly resistant to toxicity from paracetamol (acetaminophen),[57,58] which is metabolized by CYP2E1 to its reactive metabolite, resulting in a cascade of events leading to hepatic injury.

The generation of transgenic mice expressing a variety of human CYP and nuclear receptor transgenes[59-62] may help to overcome the problems encountered from genetic differences of drug metabolizing enzymes in humans and mice. Furthermore,

Table I. Examples of animal models associated with different mechanisms of idiosyncratic drug-induced liver injury

Idiosyncratic DILI hypothesis	Species (strain)	Drugs[a]	Damage	References
Metabolic activation	Rat	Paracetamol (acetaminophen) Diclofenac Flutamide Halothane Rifampicin + isoniazid	Increased serum ALT, histological hepatotoxicity, severe necrosis	18-20
	Guinea pig	Halothane	Mild to severe necrosis	21-26
Mitochondrial dysfunction	Woodchuck	Fialuridine	Increased serum AST activity	27
	Mouse (Sod2+/-)	Troglitazone Nimesulide Flutamide	Mild injury, increased number of apoptotic hepatocytes	28-31
	Rabbit	Panadiplon	Modest increase in serum ALT/AST activities, necrosis	32
	Mouse (jvs+/-)	Valproic acid	Small increases in serum ALT/ALP activities, modest histopathological changes	33
Inflammatory stress	Rat	Amiodarone Chloropromazine Diclofenac Trovafloxacin Sulindac Ranitidine	Moderate to severe necrosis, increases in ALT activity	34-39
	Mouse	Trovafloxacin Halothane	Increased hepatocyte apoptosis, severe necrosis, increases in ALT activity	40-46
Multiple determinants	Mouse	Halothane	Mild to severe necrosis, increases in ALT activity	47,48

a Listing of a drug in a particular category of idiosyncratic DILI hypothesis does not exclude the possibility that it may fit with other categories as well. Also, this is not an exhaustive list, and attempts to replicate the liver injury seen in some of these models have been unsuccessful.

ALP = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **DILI** = drug-induced liver injury; **jvs** = juvenile visceral steatosis; **Sod** = superoxide dismutase.

chimeric mice with humanized livers, via transplantation of human hepatocytes, have been generated.[63,64] Such models may provide another approach to the *in vivo* evaluation of human genetic polymorphisms although, unlike transgenic animals, they cannot be maintained by breeding.[65]

Interplay between the organic anion transporting polypeptides, Oatp1a/1b, and the sinusoidal export pump Abcc3, has been demonstrated in a *Slco1a/1b;Abcc3*$^{-/-}$ murine model. Abcc3 was shown to secrete bilirubin conjugates into the blood, while Oatp1a/1b transporters mediated their hepatic reuptake. This shuttle mechanism may have implications for modelling human idiosyncratic DILI, since the identified full-deficiency alleles of the *SLCO1B1* and *SLCO1B3* genes may contribute to various idiosyncratic drug hypersensitivities.[66]

Despite some progress, these approaches have not resulted in models in which hepatotoxicity arises from exposure to drugs that cause idiosyncratic DILI in humans. It seems likely that a drug metabolism polymorphism is often a necessary but insufficient condition for idiosyncratic DILI, so that either other cofactors or more than one polymorphism are required to result in injury. Hence, the combination of polymorphisms of a drug-metabolizing enzyme and another individual susceptibility factor may yield an animal model of DILI.

2.2 Mitochondrial Dysfunction

Mitochondrial dysfunction interrupts energy production and can eventually cause cell death and possible liver failure. It is often found in cases

of DILI, and mechanisms of drug-induced mitochondrial dysfunction (recently reviewed by Begriche et al.[67]) can be triggered not only by reactive metabolites but also by the parent drug.

Despite several studies demonstrating that numerous drugs associated with DILI can affect mitochondria *in vitro*,[68-71] animal models linking DILI to mitochondrial dysfunction are relatively few. They include hepatotoxicity of fialuridine in woodchucks,[27,65] panadiplon in rabbits,[32] valproic acid in heterozygous juvenile visceral steatosis (jvs+/-) mice[32,33] and troglitazone, nimesulide and flutamide in heterozygous mitochondrial superoxide dismutase (Sod2+/-) mice.[28,30]

However, drawbacks to these models include lack of reproducibility, species not conducive to preclinical testing, production of only modest liver injury and necessary prolonged drug exposure. Nonetheless, the idea that drug-induced mitochondrial toxicity accumulates during therapy to trigger overt hepatotoxicity is attractive, and additional effort to develop animal models could prove fruitful.

2.3 Inflammatory Stress Hypothesis

It has been proposed that a modest inflammatory episode, such as the result of a concurrent bacterial or viral infection, can interact with a drug synergistically to precipitate idiosyncratic DILI.[13,72] Exposure to an inflammagen or other stress factor may result in a shift of the dose-response curve to the left, and thus a hepatotoxic response at therapeutic doses (figure 1). This hypothesis may also explain an apparent lack of correlation of idiosyncratic DILI with drug dose, as intermittent manifestations of the stress factor could lead to continuous alterations of the dose-response curve.

Models have been developed for several drugs that cause human DILI in association with

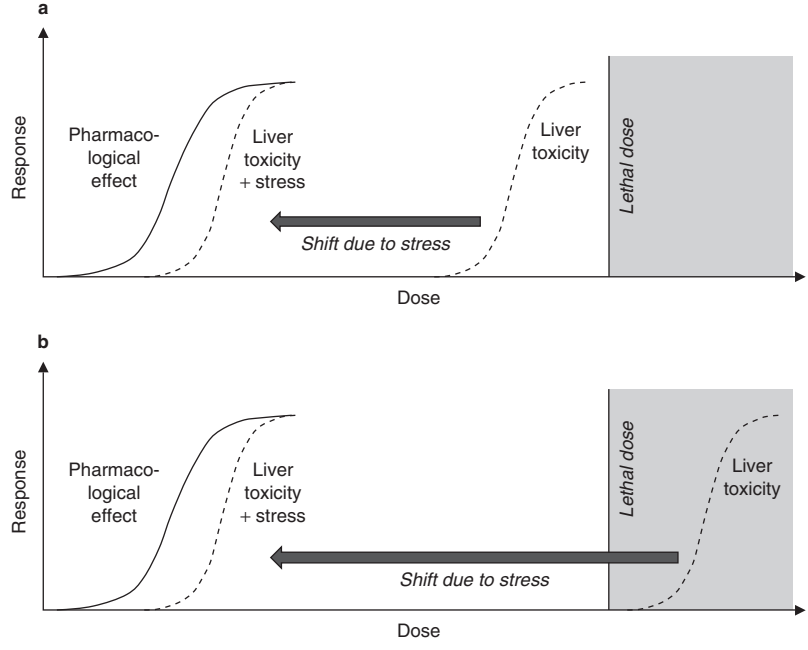

Fig. 1. Proposed dose-response curves for intrinsic and idiosyncratic drug-induced liver injury. (**a**) Susceptibility to intrinsic hepatotoxicity depends on dose, with injury occurring at smaller than lethal doses. Substantial differences in individual sensitivity to the toxic effects of the drug can occur, e.g. through stresses such as inflammation, which cause a leftward shift in the dose-response curve for hepatotoxicity. (**b**) For idiosyncratic hepatotoxicity, the dose-response curve may lie to the right of the lethal dose of a drug, so that hepatotoxicity is not seen. However, stresses may shift the dose-response curve to the left to expose hepatotoxicity within the therapeutic dose range (reproduced from Roth and Ganey,[13] with permission).

an inflammatory response (e.g. diclofenac, sulindac).[37,47,73,74] Co-treating rats or mice with these drugs and a non-hepatotoxic dose of an inflammagen such as lipopolysaccharide has resulted in pronounced liver injury. In a few cases, the approach has been able to distinguish drugs that have DILI liability from others in the same pharmacologic class that do not.

Dosing and timing of inflammagen exposure that provokes liver injury vary considerably from drug to drug, rendering current models unwieldy for preclinical safety evaluation. Nevertheless, their potential for enhancing understanding of idiosyncratic DILI-related mechanisms is considerable, and could lead to *in vitro* assays adaptable to high-throughput screening for drug candidates with DILI risk.[38,43,46,75-77]

2.4 Multiple Determinants Hypothesis

Some progress has been made in the development of animal models that incorporate the hypothesis that idiosyncratic DILI requires the combination of multiple determinants. Halothane is one of the more widely-studied drugs causing idiosyncratic DILI. A mouse model imitating severe halothane hepatitis has been developed that incorporates several human risk factors for DILI, including female sex, adult age, genetics, fasting and inflammatory stress.[47,48]

Further exploration of this type of model, which potentially best represents the complexity of idiosyncratic reactions, could increase our understanding of idiosyncratic DILI. However, this animal model may have limited feasibility until risk factors for other drugs that cause DILI are better identified.

2.5 Other Mechanisms that Currently Lack Successful Animal Models

Impaired hepatic excretory function can cause cholestasis and/or jaundice. Drugs impair this function of liver through a number of different mechanisms. There are currently no successful animal models replicating idiosyncratic DILI due to impaired hepatic excretory function, although there are animals serving as pharmacokinetic models, such as studies to investigate the effects of Gilbert's syndrome on drug metabolism.[78]

A longstanding view is that DILI can arise in some instances from an adaptive immune response to cellular proteins, which are altered by covalent binding of a reactive drug metabolite. Efforts to develop animal models of liver injury consistent with the involvement of adaptive immunity (i.e. requiring sensitization and challenge and the adaptive immune system) have not yet been successful.

Finally, the 'failure to adapt' hypothesis suggests that mild liver injury is commonplace as a result of drug therapy but, in most people, the liver adapts to the insult so that no clinically significant injury occurs. Only susceptible individuals, whose livers fail to adapt, develop idiosyncratic DILI. Thus far, no animal models based on this hypothesis have appeared using drugs that cause human idiosyncratic DILI. However, as noted above, mice given halothane under certain conditions develop modest liver injury that progresses to pronounced liver damage in the presence of a coexisting inflammatory stimulus, which can be viewed as a failure to adapt to the modest, halothane-induced injury.[40,47]

In total, the current models that have been discussed do represent progress and hold promise for increasing the prediction and understanding of human DILI in future.

3. Limitations of Current DILI Biomarkers

Besides mechanistic animal models, additional, sensitive and specific biomarkers are needed to allow the early and improved prediction of human liver toxicity in both the clinic and preclinical studies. High specificity is particularly important for detecting rare events without a high burden of false-positive test results.

The most common currently used biomarkers are alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin (TBL) and alkaline phosphatase (ALP).[10,14,79] The distinction between injury and function is important, because it is mainly when function is impaired that symptoms and clinically significant disease follow. Hepatocellular injury is generally detected by elevations in serum aminotransferase (AT)

activity, whereas serum concentrations of TBL or conjugated bilirubin are a measure of the excretory function of the liver.

Because the liver has a large excess of bilirubin-excreting capacity, hepatocyte injury must be extensive to affect the liver's ability to move bilirubin from plasma into bile. When this occurs, serum concentrations of both conjugated and, to some extent, unconjugated bilirubin are elevated with a peak that either coincides with or lags after (but does not precede) the peak of AT levels.

This represents an extent of liver damage so great that recovery may not be possible in some patients. Hence, the detection of substantially increased AT and bilirubin in the absence of cholestasis, a Hy's Law case, has become a strong predictor of whether a drug has severe DILI potential. However, there is still some debate over the most suitable Hy's Law definition, particularly the most appropriate indicator of cholestasis (table II).

The US FDA guidance on premarketing clinical evaluation of DILI states that ALP should not be substantially elevated, to rule out an obstructive basis for the elevated bilirubin. It suggests a threshold of ALP <2 × upper limit of normal (ULN) to exclude cholestasis when defining possible Hy's Law cases in new drug application (NDA) and biologics license application (BLA) submissions.[10]

This is because it is the general prevailing, though unconfirmed, view that cholestatic liver injury is associated with a lower risk of ALF than hepatocellular injury. Although an elevated ALP ≥2 × ULN has generally been used as a chemical indicator of liver injury,[12,80] the ALT : ALP ratio (R) appears to be the most appropriate criterion to characterize the clinical presentation of DILI with a value ≤2 being consistent with a cholestatic pattern of the injury.[12,77,80,81]

Data from both the Spanish DILI and the DILI Network (DILIN) registries have consistently shown that ALP >2 × ULN occurs in one-third of Hy's Law cases and it is rarely greater than 5 × ULN.[82] However, it was found that R ≥5 (hepatocellular pattern) with a TBL ≥2 × ULN at the time of ALT peak predicted the great majority of the cases leading to ALF with a fatal outcome or requiring liver transplant. Moreover, a mixed pattern of hepatocellular and cholestatic injury (R between 2 and 5) was also rarely associated with ALF and so could be considered like the cholestatic presentation with respect to Hy's Law risk.

Hence, Hy's Law could be best defined by using R to identify the hepatocellular type of injury associated with the highest risk of severe ALF. Possible criticisms of this conclusion may be that these registries do not provide sufficient data to corroborate the finding and that these data are derived from well-characterized postmarketing cases, and hence not necessarily representative of

Table II. Conditions required for a Hy's Law case

	Component	Comment
1	Elevated ALT or AST >3 × ULN	Many drugs show this signal without conferring a risk of severe injury, indicating low specificity for an excess of AT elevations alone
2	Elevated serum TBL >2 × ULN without initial findings of cholestasis	Although US FDA guidance on DILI premarketing clinical evaluation suggested ALP ≥2 × ULN as an indicator of cholestasis, an ALT : ALP ratio value ≥5 has been proposed as consistent with hepatocellular injury and may be the most appropriate discriminatory criterion[12,77,80,81] The TBL peak should coincide with or lag after the AT peak; however, in practice, serum sampling timepoints may well miss peak values
3	No other explanation for the combined increase in AT and TBL	For example: viral hepatitis (A, B or C) pre-existing or acute liver disease alcoholic or autoimmune hepatitis biliary tract disorders congestive heart failure another drug capable of causing the injury

ALP = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **AT** = aminotransferase; **DILI** = drug-induced liver injury; **TBL** = total bilirubin; **ULN** = upper limit of normal.

a clinical trial setting. However, we believe that the peak of ALT, which approximates the time of maximum injury for assessing the R value, should be readily identifiable in clinical trials where monitoring is conducted more frequently and regularly than in the real-world setting.

Another important consideration when assessing the risk of severe DILI in a premarketing clinical programme is that Hy's Law cases almost invariably arise on a background of an increased incidence of more modest signs of hepatocellular injury.[83]

There are some disadvantages to the commonly used biomarkers. AST is found not only in hepatocytes but also in skeletal muscle and heart,[84] so that damage to these tissues could give rise to a non-specific signal. Although ALT is commonly viewed as being more specific for hepatocellular injury than AST, it also occurs in many tissues.[85]

A major increase in ALT suggests cell damage, albeit ALT levels can increase without a histopathological or functional correlate.[86] Moreover, ALT only detects hepatic injury after it has occurred and, therefore, cannot be considered a true predictor. Aminotransferase measurement may also be influenced by compounds that affect the pyridoxal-5-phosphate (form of vitamin B6) pathway *in vivo*, resulting in artificially lowered ALT and AST levels.[86-89]

Elevations in serum bilirubin are most commonly due to hepatobiliary obstruction or inflammation (predominantly direct bilirubin) or to Gilbert's Syndrome, a condition of decreased activity of the uridine diphosphate glucuronosyl transferase 1A1 enzyme responsible for bilirubin glucuronidation and, therefore, characterized by indirect hyperbilirubinaemia. Likewise, indirect hyperbilirubinaemia might occur with drugs that inhibit unconjugated bilirubin transport or bilirubin conjugation in susceptible subjects or that cause haemolysis.

Nonetheless, ALT and TBL taken together become a reasonably sensitive and highly specific marker of liver injury.

3.1 Other Conventional Biomarkers

A number of enzymes associated with liver injury are measured in humans and/or other species, and have achieved a degree of acceptance based on scientific knowledge and empirical data.[90] For these biomarkers there is a weight of evidence supporting their relation with certain aspects of liver toxicity. For many of them, validated assays are available and some are also used in the clinic under specific circumstances, although they have limitations. These include glutathione S-transferase α (GSTα), gamma-glutamyltransferase (GGT), 5′-nucleotidase (5′ND), sorbitol dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase and bile acids (table III).

The more recently identified paraoxonase-1, purine-nucleoside phosphorylase and malate dehydrogenase are putative liver injury markers in the rat,[125] and some studies highlight their possible use in the clinic (table III). For these putative biomarkers, additional data are needed to support or refute their suitability in terms of specificity, sensitivity and translational use.

3.2 Genomic Biomarkers

A genomic biomarker is a measurable DNA and/or RNA characteristic, which provides an indicator for a normal biologic process, pathogenic process and/or response to a therapeutic or other intervention of interest.[126]

Transcriptional fingerprints in liver tissue can be correlated with hepatotoxicity, but are not amenable to clinical practice due to the need for fresh liver tissue. Transcriptional biomarkers have, however, proven useful endpoints for *in vitro* systems as well as predictors of hepatotoxic outcome in the preclinical setting. Also, transcriptional changes may precede the histopathological manifestation of damage so that gene expression profiling may potentially allow shorter treatment periods in animal studies and thus speed up the drug development process, e.g. in the field of hepatocarcinogenesis.[127]

Circulating microRNAs have been described as putative biomarkers for disease and toxicity, including hepatic injury in animals and man. Their non-invasive accessibility and relative stability make them attractive as potential biomarkers, and research in this area is advancing rapidly.[122,128-130]

Certain genotypes may also be considered as biomarkers if they have a strong impact on

Table III. Other biomarkers with the potential to indicate drug-induced liver injury

Biomarker	Full name	Expression	Indication	Comments	References
GSTα	Glutathione S-transferase alpha	Liver (periportal/centrilobular region) Kidney	Centrilobular liver damage	Also indication of kidney injury (through increased urinary GSTα rather than serum GSTα)	91,92
GGT	Gamma-glutamyltransferase	Liver (bile ducts) Kidney Pancreas Brain Heart Spleen	Hepatobiliary damage	Confounding factors: damage to other organs, such as pancreatitis or myocardial injury, alcohol use, drugs (phenytoin) Primary use is the exclusion of bone disease as a cause of increased serum ALP	93-95
5′ND	5′-Nucleotidase	Liver (sinusoidal/canalicular membranes) Brain Heart Blood vessels Intestine Pancreas	Cholestatic liver injury	Highly specific for cholestatic liver injury despite widespread expression	96-98
SDH	Sorbitol dehydrogenase	Liver	Hepatocellular injury	Elevated levels specific for liver involvement Less sensitive than the transaminases	99,100
LDH	Lactate dehydrogenase	Predominantly in muscle tissue Liver Kidney Brain Heart Lungs	Tissue damage	Often measured to check for tissue damage, including the liver Elevated levels of LDH-4 and LDH-5 isoforms indicate liver and/or muscle disease	101-103
GLDH	Glutamate dehydrogenase	Variety of tissues	Functional disruption of mitochondria	Activity increased in serum of patients with chronic liver disease	104
Bile acids	Cholic acid Chenodeoxycholic acid	Synthesized in liver	Functional disruption of hepatic excretory capacity	More specific indicator than serum bilirubin More sensitive to subtle excretory abnormalities than bilirubin	105-107

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Table III. Contd

Biomarker	Full name	Expression	Indication	Comments	References
PON-1	Paraoxonase-1	Liver Kidney Brain Lung	Potential marker of hepatocellular damage	Liver injury results in lower circulating levels Specificity problems reduce potential as a stand-alone marker Inconsistent results in studies	108-111
PNP	Purine nucleoside phosphorylase	Liver Heart Muscle	Potential marker of early hepatic necrosis	Relatively liver-specific Increase in serum PNP activity prior to increase of ALT in rats	112
MDH	Malate dehydrogenase	Liver Heart Skeletal muscle Brain	Potential marker of liver injury	Serum MDH activity correlates with both liver and heart injury in rats Useful in staging liver diseases clinically Increased activity in cirrhotic patients	113,114
NGAL	Neutrophil gelatinase-associated lipocalin	Variety of tissues	Potential marker of liver injury (serum)	Qualified marker for kidney injury Found to be more specific and sensitive than AST and ALT based on three hepatotoxicants	115-117
Clusterin	NA	Variety of tissues	Potential marker of liver injury (serum)	Qualified marker for kidney injury Found to be more specific and sensitive than AST and ALT based on three hepatotoxicants Has been described as a biomarker for hepatotoxicity	118-120
Thiostatin	NA	Primarily in liver	Potential marker of liver injury	Released into plasma during acute inflammatory conditions High sensitivity Specificity might be challenged as also induced in other organs	108
Cytochrome c	NA	All cells	Specific for mitochondrial injury and apoptosis	Not liver specific	121
miR-122, miR-192	MicroRNA 122 and microRNA 192	Liver	Liver specificity Sensitivity, dynamic range and half-life under investigation	Released into plasma and possibly translational as conserved across species (mouse, rat, human) Validation with regard to type of injury ongoing	122
CK-18 fragments	Cytokeratin-18	All cells	Released during hepatocyte apoptosis	Not liver specific	123
HMGB1	High-mobility group protein B1	All cells	Released during hepatocyte necrosis – acetylated form indicates inflammatory response	Not liver specific. Acetylation assay requires mass spectral techniques	123,124

ALP = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **NA** = not applicable.

responses to hepatotoxicants. The identification of specific genetic polymorphisms may highlight human subpopulations susceptible to DILI for an individual drug, and thus indicate to which patients the drug can be safely administered.[131]

These non-conventional molecular biomarkers combined with improved model systems may better predict idiosyncratic hepatotoxicity and be used as a tool to address cell type, organ and organelle-specific events.

4. Assessing DILI in Disease States with Higher Risk of Liver Disorders

The FDA guidance for industry in the pre-marketing clinical evaluation of DILI is the most specific regulatory guidance currently available on this matter.[10] It provides general recommendations on the interpretation and monitoring of DILI signals and has been useful in setting standards for the great majority of clinical indications involving subjects with a low risk of liver disorders.

On the other hand, the FDA has acknowledged that these recommendations may not be suitable for all situations, and a modified approach would be justified for special populations, such as people with pre-existing liver disease or malignancies, where a higher number of confounded DILI signals due to multiple factors could be observed.

In patients with underlying liver disease, drug pharmacokinetics is generally impaired. The effects for an individual drug are unpredictable and do not correlate well with the type of liver damage, its severity or liver laboratory test results.[132] Thus, there are no universal rules applicable across different drugs for modifying dosing regimens in patients with liver disease.[133]

The purpose of this section is to remark upon possible variations to the FDA general recommendations in circumstances where a DILI signal could be confounded by underlying diseases or concomitant drug regimens.

4.1 Viral Hepatitis

The risk of DILI and recommendations for using potentially hepatotoxic agents in patients with liver disease have been comprehensively discussed in a recent review of published data on the use of hepatotoxic drugs in chronic liver disease.[134]

Chronic liver diseases such as hepatitis B and C infection may result in AT levels that vary considerably over the course of the disease. For example, chronic hepatitis C typically results in fluctuating ALT,[135,136] and hepatitis B virus clearance is commonly associated with hepatitis symptoms and AT flares exceeding $3 \times$ ULN,[137] which can be confused with DILI. Moreover, treatment regimens for chronic hepatitis C that include ribavirin can lead to indirect hyperbilirubinaemia secondary to haemolysis. Therefore, interpretation of a potential DILI signal must take into account the time course of the background disease and/or drug regimens that highly confound DILI signals.

4.2 HIV

The diagnosis of DILI in patients with HIV can be unusually challenging, because multiple potentially hepatotoxic drugs (including reverse transcriptase inhibitors and protease inhibitors) are used as a cocktail. Additionally, HIV patients have a high incidence of underlying liver disease, including chronic hepatitis B or C, liver injury due to ethanol and illicit drug abuse, steatohepatitis due to insulin resistance, and dyslipidaemia resulting from some HIV medications.

The reported incidence of liver toxicity in HIV patients after initiating highly active antiretroviral therapy (HAART) ranges from 2% to 18%.[138] Liver toxicity, especially severe toxicity, is generally asymptomatic and clearly more frequent in hepatitis B and/or C co-infected individuals treated with HAART.[138-141] Prolonged exposure to any antiretroviral drug, co-infection with hepatitis B or C, antiretroviral therapy-naive patients undergoing their first HAART regimen, and abnormal baseline levels of ALT are all associated with a higher risk of asymptomatic hepatotoxicity.[139,142] The 'immune reconstitution' that can result from anti-HIV treatment can also cause a flare of liver injury due to a bolstered immune attack on hepatocytes chronically infected with viral hepatitis. Risk minimization is also challenging since it is

not appropriate to discontinue a single agent empirically, as this may lead to rapid development of HIV resistance.

4.3 Cancer

Mild elevations of serum AT are common among sick, febrile oncology patients taking numerous drugs. Jaundice or symptoms such as malaise, nausea, anorexia or pain also often have other causes, e.g. primary or metastatic liver carcinoma, changes in metabolic state, other treatments, malnutrition or dehydration.[143] However, patients with rapidly rising or high serum AT, or with AT elevations accompanied by jaundice, require urgent evaluation to find treatable causes of hepatocellular necrosis, although the underlying liver injury which led to jaundice may not be readily reversible irrespective of aetiology.[144]

Chemotherapy often consists of a cocktail of drugs, many of which can rapidly cause hepatotoxicity, particularly in patients with pre-existing liver impairment.[145] Drug-metabolizing enzymes and drug transporters are key determinants of the pharmacokinetics and pharmacodynamics of many antineoplastic agents, and variant metabolic pathways can significantly alter the likelihood of a toxic reaction.[145] Liver injury caused by anti-cancer drug therapies is almost always a consideration when a patient's serum AT rises following therapy, but only certain drugs and combination drug regimens are common causes of severe hepatocellular injury.

4.4 Other Disease States

Characteristics of certain other disease states may lead to a higher risk of liver disorders. For example, treatment with several non-biologic and biologic disease-modifying anti-rheumatic drugs (DMARDs) for rheumatoid arthritis often results in elevated ALT levels. AT abnormalities are more commonly associated with methotrexate-based regimens. However, no direct effect of DMARDs has been observed on bilirubin as a marker of liver excretory dysfunction.

In conclusion, the underlying disease states or associated drug regimens discussed above may lead to increased numbers of potential liver injury signals that are unrelated to the drug under investigation. It is important to be able to distinguish true DILI signals for a drug from background 'noise,' and the FDA guidance acknowledges that variations in approach may be required in certain circumstances.[10]

Therefore, depending on baseline conditions, disease development and standard drug regimens, modified sensitivity thresholds for a signal alert may be appropriate for certain disease populations. Other variations may include increasing AT thresholds for clinical trial exclusion criteria, expressing AT levels as multiples of a baseline measurement rather than of ULN for prompting closer monitoring and study drug discontinuation, and tailoring monitoring around the timing of treatment cycles.

This approach is consistent with the clinical chemistry threshold criteria recommended by the DILI Expert Working Group.[12] Modifications to the general FDA recommendations, including a customized definition of the Hy's Law case, should be discussed and agreed with health authorities as appropriate before they are implemented. Moreover, it is crucial when designing clinical trials in these confounded settings to consider control arms that are appropriately sized, to achieve sufficient power in all arms for detecting liver injury signals.

A distinction should be made between assessment of population-based signals, where a drug used in the context of underlying clinical hepatic disorder may have hepatotoxic potential masked, and the assessment of an individual clinical case where there is the propensity for underlying liver disease. In such cases, particular clinical vigilance is needed, with attention to clear clinical and diagnostic principles, and it is probably safer, if serious hepatotoxicity is suspected, to discontinue the suspected drug.

Potential drug-drug interactions (DDIs) are also an important consideration, particularly in the treatment of older patients who may have multiple chronic conditions requiring concomitant therapies. DDIs can alter a drug's toxicity profile and therefore potentially increase the risk of hepatotoxicity. The detailed assessment of drug metabolism and transport, therefore, is critical to characterize the pharmacological profile of a given drug and subsequently to manage its potential risk of liver injury in susceptible patients.

Figure 2 shows an example of a treatment algorithm with differential interpretation based on the above considerations. This type of approach could be useful to address the range and complexity of patients encountered in daily clinical practice.

5. Challenges in Identifying and Evaluating DILI in the Postmarketing Setting

Pre-approval clinical programmes are generally unable to detect rare adverse drug reactions, such as idiosyncratic hepatotoxicity, because of the limited size of the patient population studied, the strictly selected patients, and the short duration of clinical testing.

However, most recent experiences of serious DILI detected postmarketing indicate that signs of less severe liver damage (e.g. increased incidence of elevated AT) were already observed in the clinical development programme.[146-148] Hence, particular attention must be paid to such laboratory abnormalities when a drug is evaluated for approval.

Moreover, clinical trial data from products withdrawn because of DILI could be utilized to conduct more research in an attempt to understand the sensitivity/specificity and predictive values of current and new biomarkers. This will require the

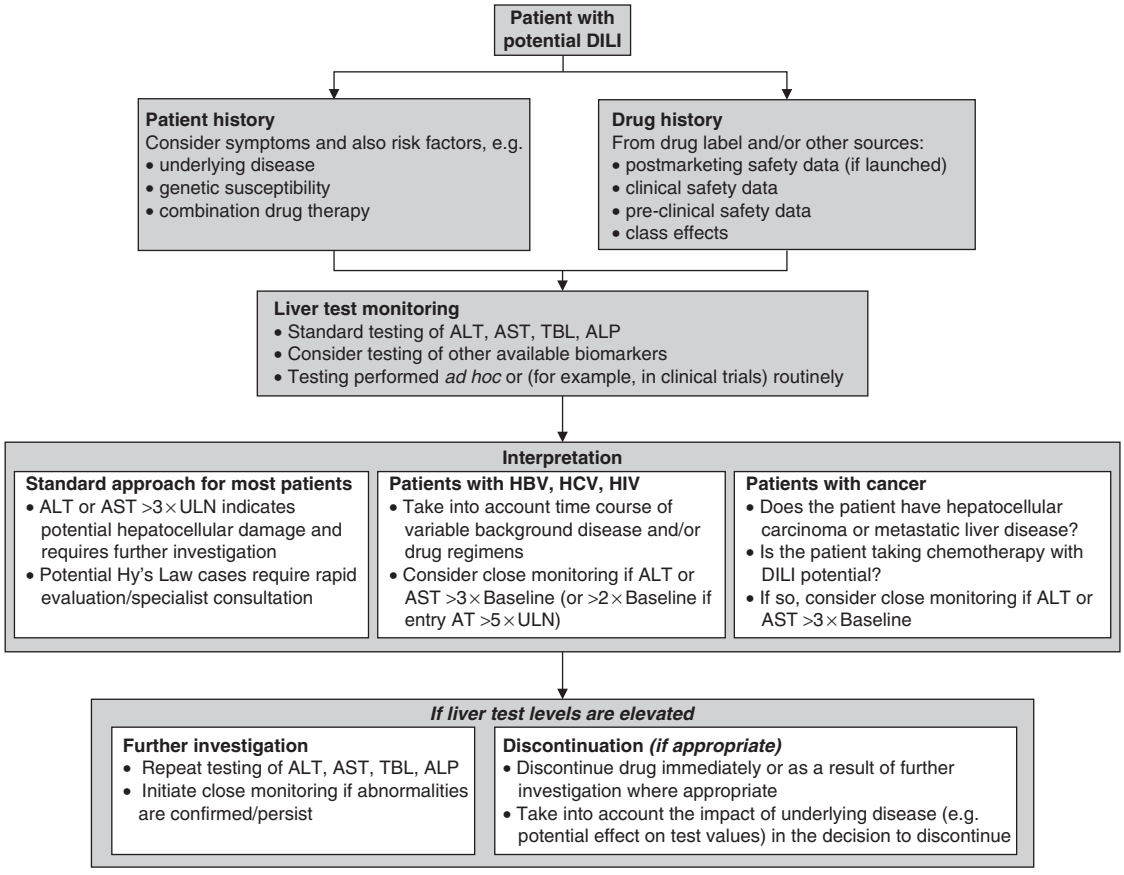

Fig. 2. Assessment of an individual clinical case for potential drug-induced liver injury. Particular clinical vigilance is needed in the assessment of individual patients with potential drug-induced liver injury where there is the propensity for underlying liver disease. This flowchart leads to a recommendation for the management of individual clinical cases, taking into consideration standard liver test results and medical background. **ALP** = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **AT** = aminotransferase; **DILI** = drug-induced liver injury; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **TBL** = total bilirubin; **ULN** = upper limit of normal.

cooperation of multiple pharmaceutical companies who may be reluctant to participate due to implications about protection of patient and company data. This concern could be assuaged by anonymized data sets, although it would reduce the types of analyses that can be performed.

An additional challenge is that liver safety data have not been stored in standardized databases, sometimes making retrieval and cross-company comparisons difficult. Going forward, it may make sense for the industry to adopt standard tools for managing and storing liver safety data, such as the eDISH format encouraged by the FDA.[149]

In comparison to a clinical development programme, the much larger number of patients taking a drug in the postmarketing setting (many of whom would not have been selected for participation in premarketing clinical trials due to stringent pre-defined inclusion/exclusion criteria), should in theory allow for easier detection of rare events such as DILI. Unfortunately, major limitations to postmarketing safety surveillance are the depth of information available to make an accurate diagnosis, which is often incomplete, the variable proportion of events reported by healthcare professionals and the lack of a control group. Another challenge is that there is no current regulatory guidance on identifying and evaluating DILI in the postmarketing setting.

Therefore, it would be wise to apply the general approach to DILI evaluation recommended in the FDA guidance for premarketing clinical trials[10] also for postmarketing trials conducted in the first few years following a drug's market entry in a new indication.

Methods to identify patients with DILI include patient registries that enrol identified DILI cases, with retrospective or prospective data collection (e.g. the DILIN). There are also efforts to identify adverse drug events in large population-based cohorts based on networks of healthcare databases (e.g. through medical billing claims or electronic health records).

However, a limitation for these approaches is incomplete or missing longitudinal follow-up data on factors needed to make an accurate DILI diagnosis. Such variables include patient risk factors for alternative causes of liver injury (e.g. hepatitis B or C), co-morbidities, medications and laboratory test results. Additionally, although there is a diagnosis code for DILI, its presence may result in over- or under-diagnosis.

The lack of a commonly agreed definition of DILI makes it difficult to identify patients when screening population-based cohorts. Even with these larger patient cohorts, DILI is so infrequent that there may be the additional need for pooling multiple health databases with different sources of data (e.g. inpatient, outpatient, laboratory test results, electronic health records).

6. Conclusions

Differing interpretations of terms such as 'idiosyncratic' and 'intrinsic' DILI have led to ambiguity and limited their value. We consider that an appropriate description of idiosyncratic DILI is drug reactions that are unexpected on the basis of the pharmacological action of the drug and only occur in a minority of susceptible individuals, compared with intrinsic DILI that results from direct toxicity of the drug, is typically secondary to drug overdose and in which most people respond in the same way.

Despite a recent proliferation of mechanisms and hypotheses on the underlying bases for idiosyncratic reactions, there are still many challenges in the prediction of hepatotoxic drugs and the assessment of DILI. There are currently few models that emulate the severe idiosyncratic hepatic injury induced by drugs in humans. Further research is needed to overcome species differences and develop models that can integrate environmental, metabolic and genetic determinants to improve understanding of DILI mechanisms and better predict which drugs will cause DILI. The current lack of a highly-specific biochemical, histologic, genetic or other type of biomarker continues to hamper the assessment of DILI and the assignment of causality to a particular agent.

A modified approach is needed for the interpretation of DILI signals for a drug under investigation in special populations with diseases having a higher risk of liver disorders or treated with hepatotoxic drug regimens. Clinical awareness and judgement in the correct diagnosis and management

of individual cases of DILI is critical, particularly when potentially confounded by underlying diseases such as hepatitis B and C, HIV and cancer. Finally, the quality, quantity and consistency of available postmarketing patient data need to improve to enhance the detection of DILI.

Addressing these challenges would facilitate the earlier identification of drugs with DILI potential and the effective management of risk, both during drug development and later in the clinic. This would ultimately lead to better outcomes for the pharmaceutical industry, for clinicians and, most importantly, for patients, whilst permitting medically useful drugs with underlying hepatotoxic potential to remain available.

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