Drug-induced liver injury: recent advances in diagnosis and risk assessment

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ABSTRACT

Idiosyncratic drug-induced liver injury (IDILI) is a rare but potentially severe adverse drug reaction that should be considered in patients who develop laboratory criteria for liver injury secondary to the administration of a potentially hepatotoxic drug. Although currently used liver parameters are sensitive in detecting DILI, they are neither specific nor able to predict the patient’s subsequent clinical course. Genetic risk assessment is useful mainly due to its high negative predictive value, with several human leucocyte antigen alleles being associated with DILI. New emerging biomarkers which could be useful in assessing DILI include total keratin 18 (K18) and caspase-cleaved keratin 18 (ccK18), macrophage colony-stimulating factor receptor 1, high mobility group box 1 and microRNA-122. From the numerous in vitro test systems that are available, monocyte-derived hepatocytes generated from patients with DILI show promise in identifying the DILI-causing agent from among a panel of coprescribed drugs. Several computer-based algorithms are available that rely on cumulative scores of known risk factors such as the administered dose or potential liabilities. The provision of more advanced scientific and regulatory guidance for liver safety assessment will depend on validating the new diagnostic markers in the ongoing DILI registries, biobanks and public–private partnerships.

IMPORTANCE OF DILI DIAGNOSIS

Drug-induced liver injury (DILI) accounts for <1% of cases of acute liver injury seen by gastroenterologists, but is the most common cause for acute liver failure in the USA and Europe.3,4 According to surveys in France and Iceland, DILI occurs with an annual incidence of about 14–19 per 100 000 inhabitants.4–6 DILI is also a leading cause of attrition of compounds in drug development and one of the two most frequent causes for drug withdrawals, restrictions and project terminations (figure 1).7–11 Of 76 drugs withdrawn from the market between 1969 and 2002, 12 were attributable to liver damage.12 Whereas liver signals that escape detection during drug approval result in postmarketing restrictions (eg, pazopanib, temozolomide and flutamide in 2013), the risk of false-positive DILI adjudication may lead to unnecessary attrition, thereby contributing to the considerable economic issues associated with DILI.

Patients who consume acetaminophen (APAP) at a single dose exceeding 7.5 g experience acute liver toxicity, notably if plasma concentrations exceed 200 or 100 μg/L 4 or 8 hours after ingestion, respectively. APAP intake at the licensed dose of 4 g/day over a period of 2 weeks results in elevations of alanine aminotransferase (ALT) above 3× the upper limit of normal (ULN) in one-third of patients.13 This form of dose-dependent APAP-induced toxicity is termed intrinsic DILI: it is predictable, reproducible in preclinical models and much insight has been gained into the underlying mechanisms.14–15

In contrast to intrinsic DILI, the onset of idiosyncratic DILI (IDILI), which is very rare but nonetheless responsible for about 10–15% of acute liver failures in the USA,16 is almost impossible to predict. IDILI is characterised by a variable latency to onset (weeks to months) and a lack of clear dose dependency.17 Drug–protein adducts, formed by drugs or their metabolites that interact with host proteins, are presented as neoantigens by major histocompatibility complex class II, thereby triggering an immunoallergic reaction. Individuals with underlying hepatic injury such as viral hepatitis or inflammatory conditions may be more susceptible to immunoallergic injury.18 Following the initial insult, additional mechanisms such as inhibition of transporters, mitochondrial injury, endoplasmic reticulum and oxidative stress and proinflammatory cytokines can further amplify the injury mechanisms that lead to acute DILI.19 Identification of host factors that render an individual susceptible is the focus of ongoing pharmacogenetic studies.20 This review article focuses on IDILI and the subsequent use of the term DILI essentially implies IDILI.

A major problem in drug development is the frequency of adverse hepatic reactions induced by the newer molecular targeted agents (MTAs) in oncology. Hepatotoxicity occurs in one-third of patients treated with a protein kinase inhibitor, with fatal outcome reported for pazopanib, sunitinib and regorafenib.21 Ten per cent of patients treated with immune checkpoint inhibitors, notably ipilimumab, develop liver injury with high rates of recurrent liver injury upon rechallenge.22 The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) gefitinib is associated with a 18.5% frequency of hepatotoxicity and casualties have occurred for all EGFR TKIs.22 The oncology population that is treated with MTAs is more likely to have multiple comorbidities and comedications and...
is therefore at risk for hepatotoxicity. The Food and Drug Administration (FDA) issues detailed recommendations in drug labels as to liver test monitoring intervals and stopping rules; however, intensive postmarketing surveillance of MTA-associated liver injury is required. The standard recommendations contained in the current FDA Guidance to Industry\textsuperscript{23} are not applicable to the oncology population and new methods of liver safety assessment are required.\textsuperscript{33,34} For the management of IDILI, a key question is whether a patient in whom DILI has been diagnosed will progress to severe liver injury with potentially fatal outcome or recover spontaneously after cessation of the causative agent. A diagnostic algorithm that allows identification of risk factors and prediction of the subsequent clinical course would greatly facilitate the management of acute DILI. The current lack of predictive safety testing before administration of a potentially hepatotoxic compound reinforces the need for rapid identification of a high-risk DILI situation that requires intensified surveillance. Novel biomarkers such as those evaluated in the European Union Innovative Medicines Initiative (IMI) Safer and Faster Evidence-based Translation (SAFE-T) Consortium have gained regulatory support for systematic implementation by sponsors in future trials.\textsuperscript{25–27}

Current diagnosis of IDILI depends on expert opinion that is based on patient data and the typical ‘signatures’ associated with certain drugs.\textsuperscript{28} Causality scores such as the Roussel-Uclaf Causality Assessment Method (RUCAM, figure 2) are intended to confirm or exclude the suspicion of DILI.\textsuperscript{29} Limitations of such scoring algorithms are poor inter-rater reliability and arbitrary scoring, for example, for alcohol use.\textsuperscript{30} This can be mitigated by a consensus process such as the one employed by the US Drug-Induced Liver Injury Network (DILIN), even though consensus opinion carries the risk of overruling a more insightful minority opinion.\textsuperscript{31,32} Liver injury caused by herbal and dietary supplements presents unique challenges to hepatotoxicity assessment and its incidence is increasing.\textsuperscript{33,34} Due to the lack of a reliable diagnostic in vitro test, there is no objective method beyond expert opinion that assesses causality of a given drug in individual cases.

**STANDARD OF DIAGNOSIS: ROLE OF CURRENTLY PERFORMED LIVER TESTS IN ASSESSING DILI**

DILI most often presents as an acute viral hepatitis-like syndrome, without symptoms that specifically point to the drug aetiology unless rash or other cutaneous manifestations\textsuperscript{35} reinforce the suspicion of drug toxicity. The clinical spectrum of DILI can mimic almost every other liver disorder. Accompanying blood eosinophilia is uncommon in large series of patients with DILI,\textsuperscript{36,37} but is clearly suggestive of drug allergy. Histopathological findings in DILI can resemble many other liver disorders, thereby limiting the value of liver biopsy in DILI diagnosis. However, biopsy can be of use to establish an alternative diagnosis when the underlying liver disease worsens (ie, alcoholic hepatitis, autoimmune hepatitis (AIH))\textsuperscript{18} (table 1).

A diagnostic workflow for assessing cases of suspected DILI is shown in figure 3. Serum aminotransferases, that is, ALT and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) levels, although not specific for DILI, remain the hallmark for detecting and classifying liver damage.\textsuperscript{40} Minor increases in aminotransferases that can result from adaptive and reversible liver responses to the drug (eg, statins) or from pre-existing liver disease (eg, fatty liver) should not be classified as DILI. An international expert group\textsuperscript{41} proposed the following thresholds for a diagnosis of DILI: (a) ALT value $\geq 5 \times$ ULN, (b) ALP value $\geq 2 \times$ ULN or (c) ALT value $\geq 3 \times$ ULN and TB $\geq 2 \times$ ULN. The latter constellation defines ‘Hy’s Law’, which anticipates a 10% risk of mortality/liver transplantation,\textsuperscript{42} as confirmed in large databases of DILI
cases. The FDA guidance for DILI extends the interpretation of Hy’s Law by stating that ‘there should not be a prominent cholestatic component’ in the hepatocellular nature of the liver injury, suggesting that a cholestatic component as defined by elevated ALP levels is associated with less risk of progression. However, a recent analysis from the Spanish DILI Registry showed that raised ALP >2 ULN did not decrease the risk of acute liver failure in cases fulfilling Hy’s Law. A marked increase of AST and an AST/AL T ratio >1.5 at DILI recognition may also predict a worse prognosis.

The presence of autoimmune features such as antinuclear antibodies (ANAs), smooth muscle antibodies (SMAs) and elevated IgG levels as well as histological features of AH may cause diagnostic confusion in DILI. Screening for autoantibodies and serum IgG in hepatocellular injury is mandatory, although the typical laboratory and pathological features of AH may also be drug induced. Moreover, recurrent DILI induced by a different drug tends to present with an AH phenotype. DILI with autoimmune features should be clearly distinguished from idiopathic AH and typically resolves after stopping the causative drug. If treated with corticosteroids, a lack of recurrence following corticosteroid cessation supports a diagnosis of drug-induced AH rather than idiopathic AH. As yet there are no diagnostic tests to differentiate idiopathic from drug-related AH, although histological findings can help in the differential diagnosis.

Rechallenge with the suspected drug, although considered the gold standard for diagnostic confirmation, carries ethical and practical issues. First, it confers a risk that is only justified when an alternative drug is not available. Second, the definition of a ‘positive rechallenge’ is controversial regarding the required threshold, if any, of liver enzyme elevation and symptoms, partly due to the lack of data on ‘negative rechallenge’. In the RUCAM score, the re-exposure test is positive if ALT is ≥2× baseline upon re-exposure, provided that ALT was below 5× ULN before re-exposure, and negative if one or both criteria are not fulfilled. Follow-up in patients with DILI must include routine liver biochemistry until complete normalisation. Rapid normalisation of aminotransferases supports the diagnosis, whereas slow or incomplete resolution suggests alternative causes. In such instances, a liver biopsy can be helpful (figure 3). Persistently elevated TB and ALP 30–60 days after the initial DILI diagnosis can predict a chronic outcome.

### IN VITRO AND IN SILICO TOOLS FOR THE ASSESSMENT AND PREDICTION OF DILI

The risk of IDILI has hung like the sword of Damocles over the drug approval process since decades, leading to fatal liver failures and subsequent market withdrawals and creating nervousness on the part of sponsors and regulators alike. These signals may occur in only very few individuals, making it impossible to identify the risk in premarketing registration trials. Thus, the quest for predictive tools that would allow an a priori identification of both host factors as well as compound features conferring a DILI risk has led to the development of numerous cell-based systems, animal models and in silico algorithms. Whereas none of these has lived up to the crystal ball promises that frequently accompany marketing initiatives, the spectrum of predictive tools available today may allow implementation of a panel of select methodologies which, in combination, yield new insight into IDILI.

Cell-based assays include primary human hepatocytes, immortalised hepatocytes, hepatoma cell lines and induced pluripotent stem cell-derived hepatocytes. These systems have been reviewed in detail and the development of coculture systems with non-parenchymal cells as well as 3D organoids has allowed...
Recent advances in clinical practice

**Figure 3** Flow diagram of diagnostic workup of drug-induced liver injury (DILI). The phenotypes of liver injury are categorised according to the Rule-of-2 which identifies potential DILI liabilities at an early stage.\(^{77}\) The predictive value of logP and daily dose could be improved, as shown in an analysis of 159 clinical cases collected from the National Institutes of Health’s LiverTox Database.\(^{72}\)

Other groups have applied inhibition of BSEP and mitochondrial toxicity parameters to the most-concern, less-concern and no-concern-DILI drugs in the LTKB and found that dual partitioning of bile acid homeostasis as mechanisms of DILI and a broad industry-wide consensus was reached on the importance of BSEP inhibition and perturbation of bile acid homeostasis as mechanisms of DILI and a broad industry-wide consensus was reached on the importance of BSEP inhibition and perturbation.

Based on the knowledge about mechanisms which cause DILI, in silico algorithms are being developed that allow modeling of various parameters to extrapolate the risk of DILI in vivo. The DILIsym model, for example, predicts that the BSEP inhibitor bosentan, but not the BSEP inhibitor telmisartan, causes mild hepatocellular ATP decline and serum ALT elevation in a simulated population.\textsuperscript{78} The catechol-O-methyltransferase inhibitors tolcapone and entacapone both cause mitochondrial impairment and inhibit BSEP, but liver injury has only been associated with the use of tolcapone. DILIsym identified patient-specific risk factors for tolcapone-induced liver injury and in a simulated population (SimPops) increases in ALT were predicted in 2.2% of the population.\textsuperscript{79} The Virtual Liver Network is a German research initiative that bridges investigations from the subcellular level to patient and healthy volunteer studies in an integrated workflow to generate validated computer models of human liver physiology.\textsuperscript{80} These in silico approaches rely on cumulative scores of known risk factors such as the administered dose or on potential liabilities such as mitochondrial toxicity, BSEP inhibition or the formation of reactive metabolites which can be measured in vitro. The major challenge when constructing predictive DILI models is to account for the broad range of chemotypes which have been associated with clinically relevant liver findings as well as the various mechanistic (pathway) considerations which translate into different clinical phenotypes of liver injury. A ‘DILI cluster score’ is being developed at Novartis that correlates a comprehensive set of several hundred compound properties against validated clinical scores as obtained from an extended version of the LTKB Database. Predictions are obtained from multiple complimentary cluster and classification models using calculated and measured compound properties related to absorption–distribution–metabolism–elimination and physicochemical properties, diverse substructural descriptors and known structural liabilities. This also allows for successful prediction of compounds which may not be classified based on typical risk factor profiles or are administered at fairly low doses (eg, methotrexate). The current algorithm is limited to orally administered drugs given over a prolonged period or in a chronic dosing regimen.

**Novel biomarkers**

There have been recent efforts mainly by public–private partnerships such as the IMI SAFE-T Consortium together with C-Path PSTC and DILIN to develop and qualify new liver safety biomarkers that outperform current standard markers in terms of sensitivity, specificity and predictivity. From the new markers investigated by IMI SAFE-T and PSTC (table 2), a subset has recently received regulatory support from both European Medicines Agency (EMA) and FDA for more systematic use in an exploratory development setting.\textsuperscript{26,27} which will ultimately

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**Figure 4**

(A) Example for a monocyte-derived hepatocyte-like (MH) cell test result from a patient with acute liver injury during treatment with sunitinib (for renal cell carcinoma), phenprocoumon (for atrial fibrillation) and metformin (for diabetes type II). MH cell toxicity is shown in a spiderweb graph. Sunitinib exerts marked toxicity in MH cells of this patient, whereas phenprocoumon and metformin do not show any effects. The red circle represents the individual cut-off for test positivity. (B) MH cell test results in 31 patients with idiosyncratic drug-induced liver injury (IDILI) and 23 patients with acute liver injury of other origin (non-DILI) using the drugs most likely to have caused liver injury in these cases. The MH cell test correctly identifies 29 of the 31 IDILI cases and shows no false-positive results. (C) MH cell test results using all drugs involved in the IDILI cases. Only four of the 84 comediations show positive results, suggesting that the MH cell test could be useful to identify the causative drug in complex IDILI cases. TWEEN, polyethylene glycol sorbitan monolaurate; ULN, upper limit of normal.
<table>
<thead>
<tr>
<th>Marker</th>
<th>Origin of biomarker</th>
<th>Summary</th>
</tr>
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<tbody>
<tr>
<td>MicroRNA (miR)-122</td>
<td>Liver specific</td>
<td>miR-122 is an early and specific marker of hepatocellular injury and a sensitive marker of DILI. It is elevated in patients who die due to liver failure.</td>
</tr>
<tr>
<td>High mobility group box 1 (HMGB1)</td>
<td>Detectable in numerous tissues</td>
<td>In acetaminophen (APAP)-induced liver injury, hyperacetylated HMGB1 is significantly elevated in patients who die or require a liver transplant, whereas in spontaneous survivors it is not significantly different from healthy volunteers. The full-length protein is released from necrotic cells. It is significantly elevated in APAP overdose patients who die or require a liver transplant compared with spontaneous survivors.</td>
</tr>
<tr>
<td>Cytokeratin 18 full length</td>
<td>Epithelial cells</td>
<td>The full-length protein is released from necrotic cells. It is significantly elevated in APAP overdose patients who die or require a liver transplant compared with spontaneous survivors.</td>
</tr>
<tr>
<td>Cytokeratin 18 caspase-cleaved fragment (caspase-cleaved keratin 18 (ccK18))</td>
<td>Epithelial cells</td>
<td>The caspase-cleaved fragment is released from apoptotic cells and helps define the type of cytotoxicity. ccK18 fragments in blood predict severity of disease in NASH and in hepatitis C.</td>
</tr>
<tr>
<td>Cadherin 5</td>
<td>Endothelial cells</td>
<td>Cadherin 5 is a calcium-dependent cell adhesion protein that is specific to endothelial cells. Initial results indicate a potential use as a susceptibility marker for DILI.</td>
</tr>
<tr>
<td>Liver fatty acid-binding protein (L-FABP)</td>
<td>Primarily liver; lower levels in the kidneys and small intestine</td>
<td>L-FABP is a sensitive marker for hepatocellular injury following liver transplantation and in hepatitis C. It may offer a better assessment of rapid changes in liver damage due to the shorter half-life of plasma L-FABP compared with ALT or AST.</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (GLDH)</td>
<td>Mitochondrial matrix; primarily in the centrilobular region of the liver; lower levels in the kidney and brain</td>
<td>A sensitive biomarker of liver toxicity with hepatocellular damage in preclinical species; shown to be elevated in humans with hepatic ischemia or hepatitis; shown to correlate with ALT in patients with a broad range of clinically demonstrated liver injuries, including APAP-induced liver injury and to detect mild hepatocyte necrosis in patients treated with heparin. Marker for mitochondrial injury or cellular injury in multiple clinical DILI and acute liver failure studies.</td>
</tr>
<tr>
<td>Glutathione S-transferase (GSTα)</td>
<td>Centrilobular region of the liver; multiple tissues</td>
<td>Hepatotoxicity biomarker shown in rats to have enhanced specificity and sensitivity compared with ALT; humans with APAP overdose show elevated GSTα levels earlier than ALT; GSTα may offer a better assessment of rapid changes in liver damage due to the shorter half-life of plasma GSTα compared with ALT or AST.</td>
</tr>
<tr>
<td>Osteopontin (OPN)</td>
<td>Multiple tissue and cell types, including liver</td>
<td>Elevated serum levels of OPN are detectable in patients with severe liver damage. Increased levels of serum OPN are associated with a poor prognosis. OPN is associated with inflammatory cell activation and liver regeneration due to activation of hepatic stem cells. Hepatocytes are a major source of OPN and HMGB1 signalling to hepatic stellate cells, thereby promoting collagen-1 production. OPN is upstream of HMGB1 and both play a major role in the pathogenesis of liver fibrosis.</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor receptor 1 (MCSFR1)</td>
<td>Cytokine receptor on macrophages/monocytes</td>
<td>Data from the ximelagatran biomarker discovery study suggest that MCSFR1 is shed from macrophages during DILI. MCSFR1 serum/plasma levels may have value as a prognostic marker for liver disease associated with inflammation.</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
<td>Multiple tissue and cell types, including liver</td>
<td>Sensitive enzymatic serum marker of liver toxicity in preclinical species. Shown to be elevated in humans with various liver diseases and to detect mild hepatocyte necrosis in patients treated with heparin. The biomarker serves two purposes: 1. as an early marker of hepatocellular injury, possibly preceding ALT on a temporal scale 2. as a specific marker of hepatocellular injury.</td>
</tr>
<tr>
<td>Bile acids</td>
<td>Synthesised by the liver</td>
<td>1. early markers of cholestasis, possibly preceding ALP and ALT on a temporal scale 2. sensitive marker of inhibition of the bile salt export pump, known to be inhibited by several drugs 3. markers of liver synthetic function.</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C-Path PSTC, Critical Path Institute’s Predictive Safety Testing Consortium; DILI, drug-induced liver injury; IMI, Innovative Medicines Initiative; SAFE-T, Safer and Faster Evidence-based Translation.
enable full qualification of the most promising markers. Once qualified in well-controlled trials, regulatory guidance will then also have to account for the new markers and incorporate them into existing guidelines.

Several new biomarkers have been studied in the context of APAP-induced DILI. MicroRNA-122 (miR-122) is a hepatocyte-specific miRNA that is elevated in the plasma of patients within hours of an APAP overdose. Together with high mobility group box-1 (HMGB1) and keratin-18, it has been shown to predict the subsequent onset of liver injury at an early time point before ALT is elevated. Previous studies in mice already showed that miR-122 and miR-192 are enriched in liver tissue and exhibit dose-dependent and exposure-dependent changes in plasma that parallel serum aminotransferase levels and the histopathology of liver degeneration.

HMGB1 is a chromatin-binding protein released by necrotic cells. HMGB1 subsequently targets Toll-like receptors and the receptor for advanced glycation end products (RAGE), thus acting as a damage-associated molecular pattern molecule. A hyperacetylated form is released from immune cells and acts as a marker of immune activation. Given the role of immune activation in IDILI, HMGB1 has been studied in the context of APAP-induced (intrinsically) DILI and as a marker of IDILI in the IMI SAFE-T Consortium DILI cohort.

Another marker of immune activation is macrophage colony-stimulating factor receptor 1 (MCSFR1). In 10 cases of IDILI caused by the centrally acting non-opioid analgesic flupirtine, the use of which has been restricted by the EMA because of hepatotoxicity, MCSFR1 levels were considerably higher than in 19 cases of APAP-induced DILI, despite ALT levels being markedly higher in APAP-induced DILI. Furthermore, both MCSFR1 and the biomarker osteopontin (table 2) were higher in 31 patients with DILI that fulfilled Hy’s Law criteria compared with 70 patients with DILI who did not fulfill Hy’s Law criteria (SAFE-T Consortium, unpublished data).

Serum bile acids have traditionally been considered to have little use in the workup of liver disease with the possible exception of intrahepatic cholestasis of pregnancy, given the multitude of analytes and the complexity of bile acid metabolism. Glycodeoxycholic acid (CDCA) has been shown to have prognostic value in predicting the outcome of acute liver failure induced by APAR with GDCA levels being considerably higher in non-surviving patients with acute liver failure (ALF). With the availability of new analytical multiplexing methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC/MS), circulating bile acid (BA) profiles are now being evaluated as biomarkers for hepatotoxicity. Targeted LC-MS/MS approach identified cholic acid, glycocholic acid and taurocholic acid (TCA) as potential biomarkers of liver injury in rodent models. In the IMI SAFE-T Consortium DILI cohort, several bile acids were markedly elevated in flupirtine-induced DILI, including glycodeoxycholic acid, taurochenodeoxycholic acid and TCA. This was not simply the result of cholestasis since ALP was markedly elevated in those cases.

In pyrrolizidine-induced liver injury, blood pyrrole–protein adducts (PPAs) predict the onset of sinusoidal obstruction syndrome with a positive predictive value of 96% and a negative predictive value of 100%. The blood PPA concentration is related to the severity and clinical outcome of pyrrolizidine alkaloid-associated hepatic sinusoidal obstruction syndrome.

GENETIC TESTING IN THE ASSESSMENT OF DILI

In keeping with their reputation as ‘hypothesis generating’ research methodology, genome-wide association studies (GWASs) have unearthed a number of novel associations, in particular between human leucocyte antigen (HLA) class I and II alleles and DILI.

In contrast to GWASs focused on other complex traits, those investigating DILI have identified risk alleles with substantially higher risk ratios for susceptibility to DILI. As demonstrated in table 3 (modified from ref. 120), there is substantial overlap among the risk alleles associated with clinically varied phenotypes of toxicities due to structurally dissimilar compounds. For example, DRB1*0701 is a risk allele for fluclaxcinil, ximelagatran and laptatinib-related DILI, while DRB1*1501 is associated with DILI secondary to amoxicillin-clavulanate and lumiracoxib. Conversely, DRB1*1501 is associated with reduced risk of fluclaxcinil DILI and DRB1*0701 is protective of amoxicillin-clavulanate DILI. Such associations extend beyond DILI into a variety of other adverse reactions, including cutaneous hypersensitivity and drug-induced pancreatitis. For example, carriage of HLA-B*5701 allele increases by 80-fold the risk of fluclaxcinil-induced DILI and the same allele is also strongly associated with hypersensitivity due to abacavir. Another example of common genetic factors underlying different organ toxicities is the link between DRB1*0701 and pancreatitis induced by thiopurine immunosuppressants as well as DILI due to a number of drugs listed above. Recently, GWASs led by the international DILI Consortium demonstrated HLA-A*33:01 as a risk factor for a cholestatic or mixed pattern of DILI when these are considered as a single phenotype irrespective of the causative drugs.

Interaction between drugs and HLA molecules

These investigations highlight the role of the adaptive immune response in DILI pathogenesis. The ‘hapten hypothesis’ states that an individual’s susceptibility to DILI is determined by the covalent binding of a drug or its metabolites to a cellular or circulating protein and the interaction of the resultant complex with the peptide-binding groove of a specific HLA molecule (figure 5). Alternatively, the ‘pharmacological interaction (p) concept’ proposes that a drug or drug metabolite can directly bind to the HLA molecule (as in the case of ximelagatran and HLA-DRB1*0701) or T cell receptor to trigger T cell activation, leading to immune-mediated liver injury. Recently, it has been proposed that drugs may make van der Waals contacts with the antigen-binding cleft (as in the case of minocycline and HLA-B*3502119) and alter the shape and chemistry of the antigen-binding cleft, thus altering the repertoire of endogenous peptides that subsequently bind to it, which in turn leads to an adaptive immune response.
Clinical applications
The majority of HLA alleles associated with DILI have a very high negative predictive value of >0.95. Therefore, genotyping can be used to rule out adverse hepatic reactions due to particular drugs (listed in table 3) so that alternative diagnoses are considered. The HLA-B*35:02 genotype is a useful diagnostic test in the setting of suspected minocycline DILI, especially in distinguishing it from idiopathic AIH as both conditions share similar serological markers such as ANAs and SMAs. A high negative predictive value of a genetic test can also be used to identify the correct agent underlying DILI when the patient has been exposed to two concomitant medications.

Overall, the strength of association between HLA genotypes and DILI has raised controversy (eg, in relation to lumiracoxib) regarding the use of genetic testing in risk stratification. The incidence of DILI is less than 1 in 10 000 for most drugs used in clinical practice and thus too low for preprescription genotyping to be cost-effective at present. It is foreseeable, however, that personal genetic information such as the HLA profile may become routinely accessible to assist precision medicine and to minimise adverse drug reactions.

EXPERT SUMMARY
DILI has raised less awareness in routine patient care than it has in the regulatory and industry setting, where DILI is a leading cause of drug attrition and a major safety issue. Acute liver failure induced by a drug in clinical practice requires immediate supportive management of the patient and referral to a liver transplantation unit if the clinical situation deteriorates. Even with a test system in place that could accurately predict a patient’s risk to develop liver failure, the likelihood that this would be routinely employed is low given the rarity of the event. This is in marked contrast to the situation in drug development, where pharmaceutical industry and regulators alike are frequently confronted with liver safety issues requiring expert assessment to quantify the risk and to implement an appropriate action scheme. Several examples of drug failures during development over the last 20 years underscore the need to develop new diagnostic tools and predictive systems which help to manage the challenge imposed by DILI.

Table 3 Genetic susceptibility for DILI identified in GWASs

<table>
<thead>
<tr>
<th>Drug studied</th>
<th>Cohort (ethnicity)</th>
<th>Association described</th>
<th>SNP (gene)</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ximelagatran</td>
<td>74 cases, 130 T controls (European)</td>
<td>HLA-DRB1*07</td>
<td>HLA-DRB1</td>
<td>4.4</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>51 cases, 282 P controls (European)</td>
<td>HLA-B*57:01</td>
<td>rs2395029</td>
<td>45.0</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>41 cases, 176 T controls; Replic: 24 cases (European)</td>
<td>HLA-DRB1*0701</td>
<td>Perfect linkage disequilibrium with DQA1*0201</td>
<td>NR</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>37 cases, 1071 T controls, (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DQB1*0602</td>
<td>3.1</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>401 cases, 532 P controls (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DRB1</td>
<td>11.3</td>
</tr>
<tr>
<td>Multiple</td>
<td>783 cases (30 diclofenac) 3001 P controls (European)</td>
<td>HLA-DRB1<em>16:01-DQB1</em>05:02</td>
<td>HLA-DRB1</td>
<td>18.7</td>
</tr>
<tr>
<td>Multiple</td>
<td>614 cases (6 flupirtine) 10 588 P controls (European)</td>
<td>HLA-DRB1*0602</td>
<td>HLA-DRB1</td>
<td>2.3</td>
</tr>
<tr>
<td>Multiple</td>
<td>862 cases (21 terbinafine; 7 fenofibrate; 5 ticlopidine cases) 10 588 P controls (European)</td>
<td>HLA-DRB1*0602</td>
<td>HLA-DRB1</td>
<td>2.8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>25 cases 10 588 P controls (European)</td>
<td>HLA-B*35:02</td>
<td>HLA-B*35:02</td>
<td>29.6</td>
</tr>
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</table>

†Predominantly.
‡Associated with diclofenac DILI only.
<table>
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<tr>
<th>Drug studied</th>
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<tbody>
<tr>
<td>Lumiracoxib</td>
<td>862 cases (6 flupirtine) 10 588 P controls (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DRB1</td>
<td>2.8</td>
</tr>
<tr>
<td>Cyclo-oxygenase-2 inhibitor</td>
<td>41 cases, 176 T controls; Replic: 24 cases (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DRB1</td>
<td>11.3</td>
</tr>
<tr>
<td>Laronidase</td>
<td>34 cases, 1071 T controls, (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DQB1*0602</td>
<td>3.1</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate antibiotic</td>
<td>201 cases, 532 P controls (European)</td>
<td>HLA-DRB1*0701</td>
<td>HLA-DRB1</td>
<td>11.3</td>
</tr>
<tr>
<td>Multiple</td>
<td>783 cases (30 diclofenac) 3001 P controls (European)</td>
<td>HLA-DRB1<em>16:01-DQB1</em>05:02</td>
<td>HLA-DRB1</td>
<td>18.7</td>
</tr>
<tr>
<td>Multiple</td>
<td>614 cases (6 flupirtine) 10 588 P controls (European)</td>
<td>HLA-DRB1*0602</td>
<td>HLA-DRB1</td>
<td>2.3</td>
</tr>
<tr>
<td>Multiple</td>
<td>862 cases (21 terbinafine; 7 fenofibrate; 5 ticlopidine cases) 10 588 P controls (European)</td>
<td>HLA-DRB1*0602</td>
<td>HLA-DRB1</td>
<td>2.8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>25 cases 10 588 P controls (European)</td>
<td>HLA-B*35:02</td>
<td>HLA-B*35:02</td>
<td>29.6</td>
</tr>
</tbody>
</table>

†Predominantly.
‡Associated with diclofenac DILI only.
Figure 5 Interaction between drugs and human leucocyte antigen (HLA) molecule leading to an adaptive immune response. (A) Hapten hypothesis: drug–protein adducts (blue circles with red semicircle) released from dying hepatocytes are phagocytosed by antigen-presenting cells (APCs) and presented with major histocompatibility complex (MHC) I molecules. These hapten–carrier complexes bind to the peptide-binding groove on T cell receptors, leading to CD4+ cell activation and an effector T cell response. (B) Pharmacological induction concept: drugs or metabolites can bind to HLA molecules directly and activate T cells. (C) Altered repertoire model: drug changes the shape and chemistry of the antigen-binding cleft, altering the repertoire of endogenous peptides that subsequently bind; the ‘altered self’ activates drug-specific T cells. (D) CD8+ cells recognise drug–protein adducts on the plasma membrane of hepatocytes when presented with MHC I molecules, leading to immunological destruction of hepatocytes.

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