Drug-induced liver injury

Raul J. Andrade[1,2], Naga Chalasani[3], Einar S. Björnsson[4,5], Ayako Suzuki[6,7], Gerd A. Kullak-Ublick[8,9], Paul B. Watkins[10,11], Harshad Devarbhavi[12], Michael Merz[8,13], M. Isabel Lucena[2,14]*, Neil Kaplowitz[15] and Guruprasad P. Aithal[16]*

Abstract | Drug-induced liver injury (DILI) is an adverse reaction to drugs or other xenobiotics that occurs either as a predictable event when an individual is exposed to toxic doses of some compounds or as an unpredictable event with many drugs in common use. Drugs can be harmful to the liver in susceptible individuals owing to genetic and environmental risk factors. These risk factors modify hepatic metabolism and excretion of the DILI-causative agent leading to cellular stress, cell death, activation of an adaptive immune response and a failure to adapt, with progression to overt liver injury. Idiosyncratic DILI is a relative rare hepatic disorder but can be severe and, in some cases, fatal, presenting with a variety of phenotypes, which mimic other hepatic diseases. The diagnosis of DILI relies on the exclusion of other aetiologies of liver disease as specific biomarkers are still lacking. Clinical scales such as CIOMS/RUCAM can support the diagnostic process but need refinement. A number of clinical variables, validated in prospective cohorts, can be used to predict a more severe DILI outcome. Although no pharmacological therapy has been adequately tested in randomized clinical trials, corticosteroids can be useful, particularly in the emergent form of DILI related to immune-checkpoint inhibitors in patients with cancer.

Drug-induced liver injury (DILI) is a term used to describe the unexpected harm that drugs in common use can cause to the liver, including damage to hepatocytes and other liver cells. The main reason explaining the susceptibility of the liver to adverse drug reactions is probably its central role in biotransformation (metabolism) of xenobiotics entering the gastrointestinal tract.

Liver toxicity related to drugs has been classically divided into two varieties based on the presumed mechanism of action of the chemical compound: intrinsic and idiosyncratic. The intrinsic (direct or predictable) type is dose-related and occurs shortly after exposure (hours to days) in most individuals exposed to the drug, which is toxic at a given threshold level. By contrast, the idiosyncratic (indirect or unpredictable) variety of DILI is determined by the interaction of environmental and host factors with the drug[1], usually occurs in <1 of every 10,000 exposed individuals, and has a longer latency period (from a few days to several months)[2]. However, clinical observations in the past decades have blurred the lines that distinguish these two types of hepatotoxicity. Unless stated otherwise, the term DILI is used for both intrinsic and idiosyncratic injury in this Primer.

The main example of intrinsic DILI is acetaminophen (also known as paracetamol or APAP) hepatotoxicity, which accounts for ~50% of acute liver failure (ALF) cases in the USA and some European countries[3,4]. Interestingly, in a substantial proportion of patients, acetaminophen hepatotoxicity occurs unintentionally at doses slightly above the maximum recommended daily dose of 4 g, which has been called 'therapeutic misadventure'[5]. Noticeably as well, in healthy volunteers alanine aminotransferase (ALT) increases frequently occur with repeated high therapeutic doses[2,6]. Supposedly, a number of factors including fasting, alcohol abuse, concomitant use of other drugs and coexisting diseases can decrease the toxic acetaminophen threshold.

Idiosyncratic DILI occurs more frequently with doses of >50–100 mg per day[7]. Hence, a minimum dose, which probably varies among individuals, also seems to be necessary to trigger the cellular cascade of events leading to idiosyncratic liver damage. Importantly, idiosyncratic DILI can be severe and, in some cases, fatal. It accounted for 11% of ALF cases in the USA in 2013 (REF.[3]), and represents a substantial concern for physicians, patients and drug companies. Because of this, idiosyncratic DILI remains a leading reason for terminating further drug development in investigational programmes, and for restrictions of use once a drug is on the market; indeed, 32% of drug withdrawals during 1975–2007 were attributed to hepatoxicity[8]. Interestingly, since publication by the US FDA in 2009 of the industry guidance document 'Drug-Induced Liver Injury: Premarketing Clinical Evaluation' and an increased awareness of DILI among the main stakeholders, no drug withdrawal notice has been issued in the USA because of post-marketing hepatotoxicity[9].

*e-mail: andrade@uma.es; lucena@uma.es

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Author addresses

[1]Unidad de Gestión Clínica de Enfermedades Digestivas, Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria, Universidad de Málaga, Malaga, Spain.
[2]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain.
[3]Division of Gastroenterology, Indiana University School of Medicine, Indianapolis, IN, USA.
[4]Department of Gastroenterology, Landspitali University Hospital Reykjavik, University of Iceland, Reykjavík, Iceland.
[5]Faculty of Medicine, University of Iceland, Reykjavík, Iceland.
[6]Gastroenterology, Duke University, Durham, NC, USA.
[7]Gastroenterology, Durham VA Medical Centre, Durham, NC, USA.
[8]Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.
[9]Mechanistic Safety, CMO & Patient Safety, Global Drug Development, Novartis Pharma, Basel, Switzerland.
[10]UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA.
[11]University of North Carolina Institute for Drug Safety Sciences, Research Triangle Park, Chapel Hill, NC, USA.
[12]Department of Gastroenterology and Hepatology, St. John's Medical College Hospital, Bangalore, India.
[13]Patient Safety, AstraZeneca, Gaithersburg, MD, USA.
[14]Servicio de Farmacología Clínica, Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria, UICEC SCReN, Universidad de Málaga, Málaga, Spain.
[15]Division of Gastroenterology and Liver Diseases, Department of Medicine, Keck School of Medicine, Los Angeles, CA, USA.
[16]National Institute for Health Research (NIHR) Nottingham Digestive Diseases Biomedical Research Centre, Nottingham University Hospital NHS Trust and University of Nottingham, Nottingham, UK.

However, complete determination of the liver safety profile of a given drug requires considerable time after drug development, usually necessitating the exposure of hundreds of thousands patients to the compound.

Many drugs in common use have been associated with hepatotoxicity events[10], although the relative risk varies widely between drugs. Drugs used in anti-tuberculosis therapy, in particular isoniazid, are the prototypical example of hepatotoxic drugs, and cause overt liver injury in 0.1–1% of individuals[11]. On the other end of the spectrum are drugs such as statins that are used to treat hypercholesterolaemia, which have been associated with hepatotoxicity in case reports and case series[12,13]. However, considering the large number of individuals exposed to these drugs, their hepatotoxic potential is very low: DILI has been estimated to occur in <1 in 50,000 treated patients[14].

The severity of DILI varies among patients, and depends on the drug type and several patient factors. Diagnosis requires a detailed clinical history, together with liver biochemistry, imaging and in some cases, liver biopsy. On the basis of results from liver biochemical tests, DILI is classified according to the pattern of liver injury, as hepatocellular, cholestatic or mixed. In general, most patients make a full recovery, although some develop ALF and may require liver transplantation, whereas others may develop chronic DILI. Although research has provided new data on DILI epidemiology and has led to a better understanding of its pathogenesis, substantial gaps still remain, particularly in the field of DILI prediction, diagnosis and therapy.

This Primer discusses the epidemiology, mechanisms, diagnosis, screening, prevention and management of DILI, including aspects of quality of life (QOL) and the outlook, highlighting areas for future research.

Epidemiology

Determining the true incidence of DILI worldwide is difficult given the diverse cultures, traditions, health-care systems and lack of consistent reporting systems and definitions. No studies have specifically analysed trends in the incidence of DILI over time. Although two on-going prospective studies in Spain and in the USA have not demonstrated any major differences in the prevalence of DILI over time,[15,16] these studies are not population-based and, therefore, do not enable analysis of the changes in the incidence over time. However, in follow-up studies, the proportion of patients in whom DILI was caused by herbal and dietary supplements has been increasing[17,18]. Furthermore, increased use of biological agents such as infliximab has been associated with an increasing frequency of DILI that is associated with these agents[13].

Asia

The only prospective nationwide study of DILI in Asia was undertaken in South Korea over a 2-year period in 17 referral university hospitals. This study reported an extrapolated incidence of hospitalization because of DILI of 12 cases per 100,000 persons per year[19], the most common causes of which were traditional and herbal medicines, which were implicated in >72% of cases.[19] A retrospective study in China found an estimated annual incidence of 23.80 cases per 100,000 persons in the general population, which is much higher than that reported in western countries.[20] Traditional medicines are often integrated into the health-care systems of technologically well-advanced countries in Asia, such as South Korea and Singapore[21]. In Japan, although traditional and herbal medicines are less integrated into the health-care system, the incidence and proportion of DILI caused by traditional medicines is increasing[22]. The proportion of DILI caused by traditional medicines and dietary supplements varies substantially across countries in Asia, with 15% in Japan[22], ~27% in China[23] and 71% in Singapore[24]. In both China and India, the incidence of DILI caused by traditional medicines is increasing[25,26].

In India and China, anti-tuberculosis drugs are the most common and the second most common causes of DILI[23,27], respectively. Indeed, in India, DILI caused by anti-tuberculosis drugs is a leading cause of ALF, which is not surprising given that 22.7% of individuals with tuberculosis worldwide live in India[28], and given the hepatotoxic potential of three of the four first-line anti-tuberculosis drugs (isoniazid, rifampicin and pyrazinamide).[29]

Europe

The annual incidence of non-fatal DILI was 2.4 cases per 100,000 persons in a retrospective study of the General Practice Research Database (GPRD) in the UK[30]. In this study, 1,636,792 individuals registered in the GPRD database were followed for 5,404,705 person-years, and 128 patients were subsequently deemed to have developed definite DILI based on retrospective causality assessment of their medical records[30]. In a retrospective analysis of

1,164 patients with liver disease at an outpatient hepatology clinic in Sweden, 6.6% had at least possible DILI[31]. These data were extrapolated to estimate a crude incidence of DILI of 2.3 per 100,000 individuals per year, with a main cause of antibiotics[31]. An annual crude incidence of ~14 cases per 100,000 inhabitants was reported in a population-based, prospective study of >81,000 individuals in France[32]. By comparison, the annual incidence of DILI was 19 cases per 100,000 inhabitants in a more-recent prospective, population-based study in Iceland[13]. Similar to other cohort studies in Europe[16,33], antibiotics were the most common drug class and amoxicillin–clavulanate was the most common single agent to cause DILI; 1 in 2,350 users of amoxicillin–clavulanate were affected in the Icelandic study[13].

USA

In a study investigating the incidence of idiosyncratic DILI in the USA based on individuals presenting with suspected DILI to gastroenterologists in Delaware, 20 individuals met the definition of DILI in 2014, which yielded an annual incidence of 2.7 cases per 100,000 adults[34]. In 14 individuals who were further characterized, DILI was attributed to the use of prescription medications in 8 individuals (57%; antibiotics in 36%) and to the use of herbal and dietary supplements in 6 individuals (43%)[34]. Another study investigated the population-representative incidence of drug-induced ALF in Kaiser Permanente (an integrated healthcare system) in northern California[35]. Although acetaminophen was the most common cause of drug-induced ALF (56% of cases) in this study, the incidence of ALF caused by idiosyncratic DILI was 1.02 (95% CI 0.6–1.6) cases per 1,000,000 person-years. Herbal and dietary supplements were a more common cause of ALF than prescription medicines in this study.

Other regions

In 2011, a multinational prospective Latin American arm of the US DILI Network (DILIN) was set up, bringing together hepatologists from ten countries. This initiative follows the same structured protocol and adjudication criteria as the Spanish DILI Registry. In this network, amoxicillin–clavulanate was the most common cause of DILI among the 330 patients with well-phenotyped DILI, 60% of whom had hepatocellular injury, which is similar to findings from other prospective DILI registries. However, nitrofurantoin and cyproterone acetate were also common causes of DILI, reflecting the differences in pharmaceutical policies and patterns of drug use across countries[36]. In sub-Saharan Africa and other resource-limited regions, traditional remedies are the main source of pharmacological care, but data on hepatotoxicity are scarce and are mainly related to anti-tuberculosis drugs in patients with HIV infection[37].

Mechanisms/pathophysiology

Normal drug metabolism and transport

The liver is an important target for drug toxicity because of its important role in removing drugs, especially lipophilic agents, from the circulation. The process of drug uptake into hepatocytes, drug metabolism and elimination is controlled by large families of proteins the individual expression and functions of which are controlled by genetic and environmental factors, including drug–drug interactions and concomitant disease, which collectively influence the accumulation of (exposure to) drugs and their metabolites and lead to stress-promoting effects of drugs in the liver[38]. Drugs are taken up into hepatocytes passively or by an array of transport proteins located in the basolateral plasma membrane (FIG. 1). These transport proteins include members of the solute carrier (SLC) family, the organic anion transporting polypeptide (OATP) superfamily [39], the organic anion transporter (OAT) family[40] and the organic cation transporter (OCT) family.

After uptake by hepatocytes, drugs are metabolized by phase I and phase II enzymatic reactions. After phase I reactions, the metabolites usually have only minor structural differences from the parent drug but can have very different pharmacological actions. Phase II metabolism involves the conjugation of a drug or metabolite with endogenous molecules such as glucuronic acid, sulfate or glutathione resulting in a more polar product that usually does not have pharmacological activity. Drugs and metabolites efflux from hepatocytes into the bile or back into the sinusoidal blood for subsequent renal excretion, which is mediated mainly by ATP-binding cassette (ABC) transporters such as multidrug resistance protein 1 (MDR1), also called P-glycoprotein, which is encoded by *ABCB1*, and anion exchange mechanisms.

Hepatotoxic substrates and metabolism

Human hepatocytes express the transporters OATP1B1 (encoded by *SLCO1B1*), OATP1B3 (encoded by *SLCO1B3*) and OATP2B1 (encoded by *SLCO2B1*)[41]. Statins are potentially hepatotoxic substrates and plasma statin levels — a risk factor for statin-induced myopathy — increase in the presence of OATP1B1 inhibitors such as cyclosporine A (an immunosuppressant) or gemfibrozil (a lipid-lowering agent)[42,43]. The main consequence of drug-induced inhibition of an uptake transporter is the effect on the pharmacokinetics of other drugs that are taken up by the same transporter, the plasma levels of which can increase due to delayed hepatic clearance. Several tyrosine kinase inhibitors (TKIs, which are small molecules used to treat various forms of cancer) confer a risk for hepatotoxicity. One example is the TKI pazopanib, which has a box warning for hepatotoxicity from the FDA. Pazopanib, like other TKIs[44], is a strong inhibitor of OATP1B1 but is taken up into hepatocytes by OCT1 (encoded by *SLC22A1*)[45]. Whereas inhibition of drug efflux transporters can lead to the accumulation of potentially toxic metabolites within hepatocytes, inhibition of basolateral uptake transporters would not be expected to be a mechanism of hepatotoxicity. The FDA provides further guidance on in vitro metabolism-mediated and transporter-mediated drug–drug interaction studies with investigational drugs (see REF.[46]).

One possible mechanism of DILI is the formation of reactive metabolites during phase I and II reactions[47]. Indeed, the covalent binding of reactive metabolites to cellular proteins can lead to alteration of the function

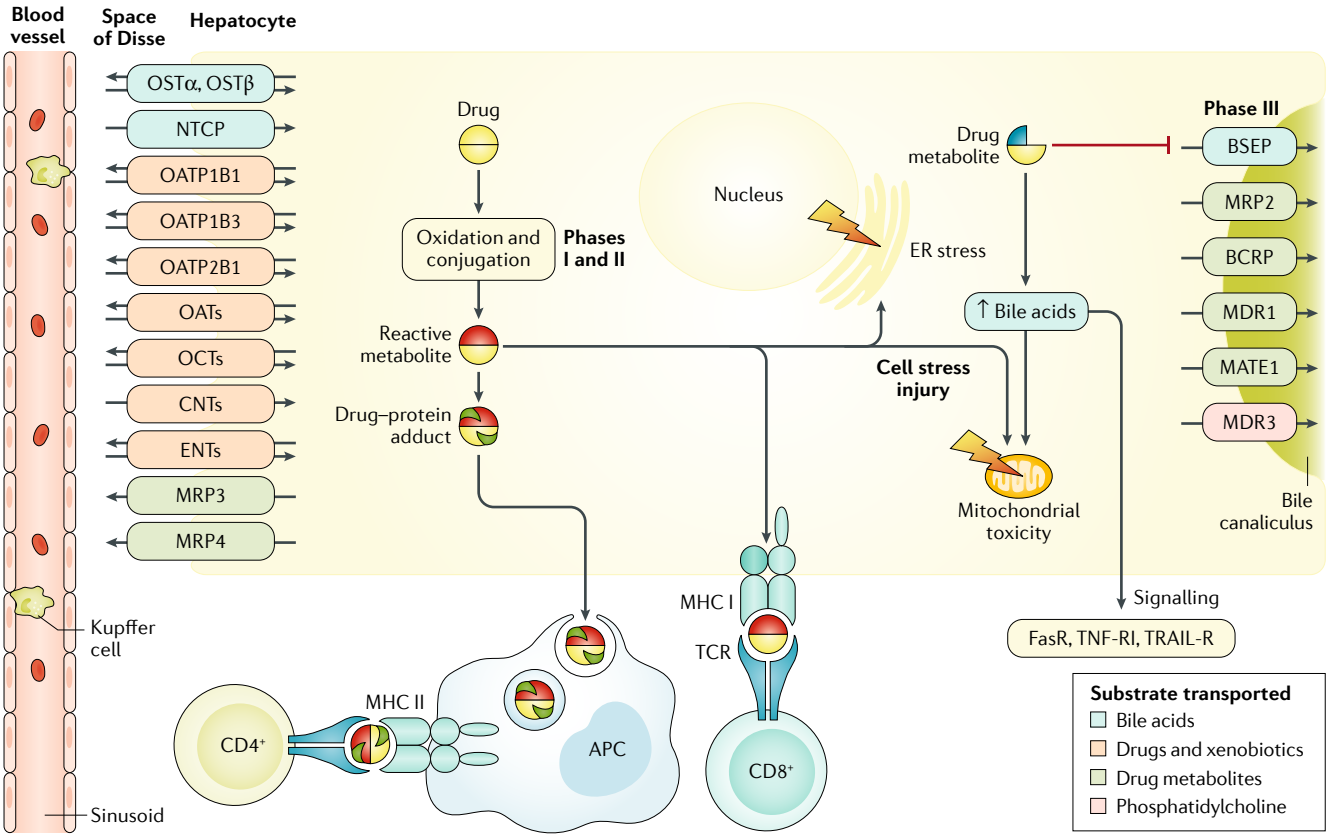

Fig. 1 | **Hepatocyte transporters and cellular mechanisms of DILI.** Blood plasma enters the perisinusoidal Space of Disse through the fenestrated liver sinusoidal endothelium and is in direct contact with the basolateral surface of hepatocytes. Drugs are taken up from sinusoidal blood into hepatocytes via transporters located in the basolateral membrane. These transporters include members of the organic anion transporting polypeptide (OATP), organic anion transporter (OAT) and organic cation transporter (OCT) families. The process of drug metabolism and elimination in hepatocytes occurs in three phases. During phase I reactions, drugs are metabolized by cytochrome P450 enzymes, a process that can generate reactive oxidative metabolites that are potentially toxic to the cell through covalently binding to cellular proteins (forming a drug–protein adduct), thereby inhibiting the function of the protein, or by causing cell stress. Drug metabolites are conjugated to endogenous molecules (phase II), following which, they are eliminated from the cell via ATP-dependent efflux pumps such as the multidrug resistance gene product (MDR), this efflux process representing phase III of drug metabolism. Cell injury releases drug–protein adducts that can act as neoantigens, triggering an immune response in susceptible individuals. Drug metabolites can also inhibit the hepatocyte canalicular efflux transporters such as bile salt export pump (BSEP), causing an increase in intracellular bile acid concentrations that damage mitochondria and lead to hepatocyte death. Bile acid-induced stress can also lead to increased targeting of death receptors (such as tumour necrosis factor receptor (TNF-R) and FasR) to the plasma membrane and sensitize the cell to ligand (such as TNF or FasL)-induced apoptosis or necrosis, or can induce ligand-independent activation of death receptors. Drug-induced liver injury (DILI) can be frequently caused by a combination of intrinsic mechanisms such as inhibition of BSEP and mitochondrial toxicity, with subsequent immune damage to hepatocytes. APC, antigen-presenting cell; BCRP, breast cancer resistance protein; CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; ER, endoplasmic reticulum; MATE, multidrug and toxin extrusion transporter; MHC, major histocompatibility complex; MRP, multidrug resistance-associated protein; NTCP, sodium/bile acid cotransporter; OST, organic solute transporter; TCR, T cell receptor; TNF-RI, TNF receptor 1; TRAIL-R, TRAIL receptor.

or location of the target protein, or to the formation of immunogenic haptens, which can trigger an immune response[48] (FIG. 1). For example, the NSAID diclofenac can cause severe hepatotoxicity and has been shown to form reactive quinone imines and acyl glucuronides during phase I metabolism[49]. In addition, lumiracoxib and troglitazone, both of which caused fatal hepatotoxicity that led to market withdrawal, form reactive quinine metabolites[50,51]. In general, drugs metabolized by the cytochrome P450 enzymes CYP2C9, CYP1A2 and CYP3A4 have a higher likelihood of forming reactive metabolites and inducing hepatotoxicity than drugs that are not metabolized by these enzymes[52].

Another possible mechanism of DILI is inhibition of the bile salt export pump (BSEP, encoded by *ABCB11*)[53], which leads to increased intracellular concentrations of bile salts that can damage mitochondria[54], leading to cytotoxicity and liver injury[55]. Potent BSEP inhibitors include bosentan (which is used to treat pulmonary hypertension and has a box warning for hepatotoxicity) and cyclosporine A, which can lead to drug-induced cholestasis[56–58]. In addition, the major metabolite of

the diabetes hepatotoxic drug troglitazone — that is, troglitazone sulfate — competitively inhibits BSEP and accumulates in hepatocytes, leading to an increase in intracellular bile salt concentrations and mitochondrial damage[59]. Some evidence suggests that drugs that inhibit BSEP are potentially more likely to cause idiosyncratic DILI than drugs that do not inhibit BSEP. This finding has led to the hypothesis that the retention of bile acids in hepatocytes can induce cellular stress. Furthermore, bile acids can induce hepatocyte apoptosis through increased plasma membrane targeting of death receptors, which can cause apoptosis via ligand-independent activation or ligand-dependent (mediated by tumour necrosis factor (TNF), FasL and TRAIL) mechanisms[60]. As conjugated anionic drug metabolites are substrates of multidrug resistance-associated protein 2 (MRP2)[61], genetic variants of this transporter have been associated with DILI[49,62,63]. In addition, genetic variants of *ABCG2* are associated with hepatotoxicity induced by the TKI sunitinib; these variations lead to reduced transport activity of ABCG2, thereby leading to intracellular accumulation of sunitinib[64].

Dysfunction of the multidrug resistance gene product 3 (MDR3, encoded by *ABCB4*), which translocates phosphatidylcholine from the inner to the outer leaflet of the lipid bilayer, is associated with various forms of cholestasis[65]. Phospholipids are an essential lipid component of bile that solubilize cholesterol in phospholipid–cholesterol vesicles. In addition, phospholipids are believed to protect cholangiocytes from bile acids by keeping them in micelles, and the 'naked' bile acids are believed to damage cholangiocytes and cause cholestatic or mixed injuries. MDR3 is inhibited by the antifungal agent itraconazole, among other drugs, resulting in reduced phospholipid output into bile[66]. Damage to cholangiocytes and small bile ducts can impair bile flow, leading to hepatocellular retention of cholephilic compounds and cholestatic liver injury. Antifungal azoles also inhibit BSEP and the combined inhibition of MDR3 and BSEP represents a dual mechanism by which azoles cause DILI in susceptible patients.

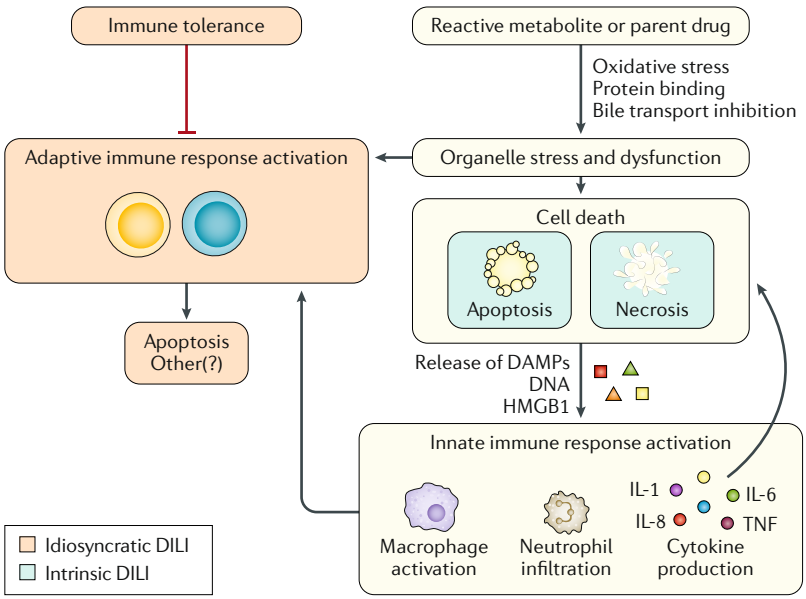

Fig. 2 | **Molecular mechanisms of idiosyncratic and intrinsic DILI.** Drug-induced liver injury (DILI) is most often caused by lipophilic drugs, which are converted to reactive metabolites that have the potential to covalently bind to proteins, leading to cellular organelle stress. The reactive metabolite might target mitochondrial or endoplasmic reticulum (ER) proteins and induce mitochondrial or ER stress, which promotes organelle-specific adaptive responses to increase chaperone proteins that protect against misfolding in organelles or antioxidant response through gene regulatory programmes triggered by redox-activated transcription factors (such as Nrf2). When the adaptive responses are inadequate, hepatocyte death occurs mediated either by collapse of mitochondrial function (mitochondrial membrane transition pore) and necrosis, or by activation of regulated cell-death pathways. The regulated cell-death pathways involve permeabilization of the outer mitochondrial membrane due to activation of pore-forming proteins such as Bax, Bak and Bid, leading to release of cytochrome *c*, caspase activation and apoptosis. Other programmed cell necrosis mechanisms might, in theory, contribute to DILI, such as necroptosis or pyroptosis, which permeabilize the cell membrane, or ferroptosis, but remain unproven. Organelle stress can release damage-associated molecular patterns (DAMPs) such as high mobility group box 1 protein (HMGB1) or DNA, which activate Toll-like receptors and lead to pro-inflammatory cytokine or chemokine release. The inflammatory response amplifying cell death in intrinsic DILI, depending on the acuity and severity of injury, may promote resolution. By contrast, the innate immune response might provide danger signals to amplify adaptive immunity in idiosyncratic DILI. The key is that polymorphisms in HLA (encoding the major histocompatibility complex (MHC) proteins), which favour the presentation of drug-adducted peptides, can be HLA-restricted so that individuals carrying the HLA variant are mainly susceptible to developing an adaptive immune response, typically leading to a T cell response directed at hepatocytes and usually involving cytotoxic CD8 T cells that target the peptide drug exposed on MHC class I molecules on the hepatocytes. However, this process can sometimes lead to antibody-dependent cytotoxicity. It is proposed that most patients who have a genetic HLA predisposition do not experience significant injury because most develop immune tolerance. Thus, progression to overt DILI is speculated to be due to impaired immune tolerance.

Adaptation and injury progression

Most of the insights into mechanisms of cell death in DILI are based on rodent and human hepatocyte culture studies, as well as in vivo studies in animal models, and focus on the pathogenesis of intrinsic DILI. Human studies mainly involve biomarkers and genetic influences on susceptibility to DILI.

Intrinsic DILI generally refers to direct toxic stress leading to cell death of hepatocytes (sometimes sinusoidal endothelial cells are the principal target) that is mediated by a reactive metabolite or a parent drug interfering with specific cell functions. This toxicity can be mediated by increased oxidative or redox stress, mitochondrial dysfunction, endoplasmic reticulum (ER) stress or DNA damage[2,38,67,68]. As these processes progress unchecked, cell death occurs (FIG. 2). Innate immune responses that are initiated by damage-associated molecular patterns (DAMPs) released from stressed or dying hepatocytes, lead to activation of resident liver Kupffer cells, natural killer and natural killer T cells, which produce cytokines and chemokines including TNF, IL-1β, IL-8, IL-6 and CXCL10 that infiltrate leukocytes. This process may add further injury to the liver[2,69]. However, there is considerable controversy about the role of the innate immune system in acute DILI in animal models, which may be due to the rapid progression of injury seen in these models in comparison to humans in whom injury progresses more slowly[70,71].

The hepatocyte toxicity in DILI is considered to be a result of mainly regulated modes of cell death, predominantly necrosis and apoptosis[72]. A final pathway leads to

complete collapse of mitochondria by increased permeability of the inner and outer membranes. The mitochondrial membrane transition pore complex is dysregulated by stress signal transduction (via MAP kinase), which amplifies the direct effects of toxic metabolites in mitochondria (such as acetaminophen toxicity)[67]. Alternatively, the intrinsic stress can activate initiator caspases (such as caspase 8) and Bcl family (such as Bid or Bax) proteins, which selectively permeabilize the outer mitochondrial membrane, releasing cytochrome *c* that activates executioner caspases (such as caspase 3 and caspase 7)[68]. Furthermore, DAMPs released from injured hepatocytes activate innate immune responses, including cytokines such as TNF and FasL, and TRAIL-expressing natural killer or natural killer T cells and neutrophils, which can activate death receptors such as TNF-R, FasR and DR5. Aside from acetaminophen toxicity, which is largely mediated by necrosis, the relative contribution of necrosis and apoptosis is largely undetermined for other drugs that can cause intrinsic DILI. Alternative mechanisms of regulated necrosis have emerged in recent years, such as necroptosis, pyroptosis and ferroptosis, although whether these are relevant to acute or chronic DILI is unknown, but they might be important in nonalcoholic steatohepatitis (NASH) or alcoholic steatohepatitis(ASH) and autoimmune hepatitis (AIH)[73]. In acute DILI, the weight of evidence indicates that necroptosis (RIPK3/MLKL-dependent cell death) has no or only a minimal role, probably because RIPK3 is not expressed under basal conditions[67].

Another potential factor in the pathogenesis of DILI is the influence of the gut microbiome, which could influence the enterohepatic circulation of drug metabolites or the status of drug metabolism through effects on the susceptibility to DILI or effects on the innate immune system. Although understanding the influence of the microbiome in DILI is in its infancy, two animal studies have illustrated the potential importance. Indeed, one study showed that tacrine hepatotoxicity is enhanced by deconjugation of tacrine glucuronide, leading to reabsorption of the parent drug, thereby exposing the liver to more tacrine through enhanced enterohepatic cycling of the parent drug[74]. In addition, the other study demonstrated that the diurnal variation of the gut microbiome determines diurnal susceptibility to acetaminophen hepatotoxicity, possibly by distinct exposure of the liver to bacterial metabolites[75]. Although these studies have focused on intrinsic DILI, the influence of the microbiome on idiosyncratic DILI is unexplored and is of great interest.

In contrast to intrinsic DILI, idiosyncratic DILI occurs in a small proportion of patients exposed to a drug, reflecting the important contribution of host genetic and environmental factors. The preponderance of evidence is that idiosyncratic DILI is usually dependent on the adaptive immune response of the individual, which is determined by HLA polymorphisms and other contributing factors that determine neoantigen (hapten peptide) presentation[76,77]. However, although unique HLA types seem to be important determinants of the immune response to reactive metabolites or parent drugs in some cases, most people with a specific drug-related risk HLA haplotype are unaffected by exposure to the drug, suggesting that other factors are involved. The identity of the other factors is not well defined. However, the extent of underlying drug-related toxic cellular and subcellular stress may be upstream (co-activator) of the development of an adaptive immune response.

As previously mentioned, idiosyncratic DILI has its onset after a variable but sometimes long latency (generally <6 months) and is not dose-independent but mainly occurs with doses of drugs >50–100 mg per day[78]. This feature probably reflects the fact that there is a threshold for activation of the immune system. High daily doses of a drug, and its metabolism in the liver are key factors in achieving a sufficient toxic exposure of hepatocytes to the drug, which is a prerequisite for most DILI cases. The adaptive immune system has a major role in the pathogenesis of idiosyncratic DILI. The adaptive immune system can be activated by haptens leading to restricted presentation of a peptide adduct by the major histocompatibility complex (MHC) proteins encoded by HLA. In rare cases, a drug might directly bind to certain MHC molecules or T cell receptors and activate an immune response (FIG. 1). Alternatively, in some instances a drug or metabolite may alter the MHC binding groove leading to misdirected peptide presentation.

A potential unifying aspect of both intrinsic and idiosyncratic DILI has been demonstrated using in vitro systems[79]. These test systems have been used to identify toxic stress in the absence of immune activation, and have indicated that hepatocyte stress and covalent binding of drug metabolites to proteins promotes neoantigen (hapten) formation and/or generates signals that co-activate the immune response.

One important modulating factor in idiosyncratic DILI is the interaction between the onset of immune activation and the participation of immune tolerance. Several examples of the importance of immune tolerance have been demonstrated in recent mouse studies in which the inhibition of several of the key mediators of immune tolerance have unmasked liver injury, as well as actually worsening DILI from its onset[80,81]. The proof of this mechanism in humans is lacking but it probably exists. Drugs that are used to break immune tolerance for cancer treatment can lead to an autoimmune-like acute injury to the liver by eliminating the immune privilege, which is characteristic of the liver. Thus, the near universal stress in the liver from parent or metabolized drugs given at or above the dose threshold, could be speculated to cause liver injury that is either below the threshold for detection or associated with mild ALT increases that disappear with continued exposure to the drug. Thus, adaptive responses, which dampen the initial toxic stress, or the development of immune tolerance might inhibit progression to overt liver injury. In theory, this adaptation could begin before any sign of liver injury appears (such as increases in ALT levels) or after the initial immune-mediated liver injury is detected (delayed asymptomatic increases in ALT levels that resolve despite continued treatment with the offending drug), referred to as clinical adaptation. Accordingly, overt liver injury could be a failure of immune tolerance[81]. Though somewhat speculative, this hypothesis is plausible and provides a framework for future studies.

Interaction between drugs and patient factors

Specific drug properties, such as high daily recommended doses, BSEP inhibition, reactive metabolite formation, mitochondrial toxicity and induction of oxidative stress, have been associated with drugs that have hepatotoxic potential in humans[1]. In addition, patient factors can predispose to DILI, including older age, multiple drug use and genetic variants[13,30,82]. However, each of the elements alone does not accurately dictate the risk of DILI in humans, corroborating the multifactorial nature of this disease. As detailed in the above sections, mechanisms involved in DILI are multiphasic. Early phases (up to the initiation of cellular damage) are more drug-specific and are primarily influenced by drug exposure (for example, dose or duration) and certain drug properties. By contrast, later phases are defined by how the patient responds to toxic stress and induces orchestrated cellular adaptation, immune responses and tissue repair processes. Drugs and patient factors influence multiple mechanisms and, therefore, probably interact at different levels, defining DILI risks, clinical phenotypes and outcomes in a sophisticated manner[1] (TABLE 1). One notable example of drug–patient interactions is with acetaminophen. Fasting and alcohol intake, among other factors, can lower the toxic dose of acetaminophen by increasing the generation of reactive drug metabolites via CYP2E1 and/or by depleting the hepatic glutathione concentration, which is the main detoxification pathway for acetaminophen toxic intermediates[2].

Few experiments have investigated the drug–patient interaction in DILI. Sex differences have long been recognized at the biochemical and cellular levels[83]. Cryopreserved primary hepatocytes derived from men and women respond differently to various toxic compounds, suggesting a drug–sex interaction at the cellular level[84]. Another study using freshly isolated primary human hepatocytes did not show sex differences in ALT elevation following acetaminophen exposure although the study suffered greater inter-individual variance probably due to the diverse clinical conditions of the donors (for example, metastatic colon cancer, head injury, stroke, drug overdose and cardiac arrest), a wide range of donor ages and the lack of consideration for menopausal status.[85] In addition, in animal studies, sex differences in susceptibility to DILI depend on the model used: a male dominance in liver injury induced by acetaminophen[86–88] and cocaine (only after the onset of puberty) has been observed[89,90], with female dominance with halothane[91–94] and in another immune-mediated DILI model[95]. In humans, age, sex and a proxy for menopausal status in women(50 years of age) significantly influence drug-specific reporting frequencies of liver events in the WHO VigiBase™ database[96,97], and influence clinical and histological phenotypes of DILI[98,99]. Drugs associated with sex-biased or age-biased reporting frequencies of liver events show distinct properties[96]. For example, drug properties such as an association with mitochondrial toxicity, reactive metabolite formation and BSEP inhibition are more prevalent among drugs with women-biased reporting frequencies[96]. High lipophilicity, biliary excretion, higher transporter inhibitions, a higher C_{max} (the maximum serum concentration that a drug achieves) and plasma protein binding, yet shorter plasma elimination time are more prevalent among drugs with age-biased reporting frequencies[96,97]. In addition, drug properties, patient factors and their specific interactions can influence the likelihood of delayed onset of DILI[100].

Despite the scarcity of the data, emerging evidence suggests the significance of considering both drug and patient together in assessing DILI risk. Future methodological implementation to cope with the complexity in DILI mechanisms and new human data sources that provide sufficient size and statistical power to address drug-specific DILI risk factors and drug–patient interaction (such as big data analysis) are needed.

Diagnosis, screening and prevention

Clinical phenotypes and case characterization

The clinical manifestations of DILI are heterogeneous. Indeed, DILI can mimic acute and chronic liver diseases of various aetiologies, and symptoms can include fever, nausea, vomiting, jaundice, dark urine, itching and right upper quadrant pain. Certain drugs have signature injury patterns (such as acetaminophen, amiodarone, diclofenac and isoniazid for hepatocellular injury, and anabolic steroids, captopril and erythromycin for cholestatic injury) but others, such as atorvastatin, allopurinol and amoxicillin–clavulanate, have various DILI manifestations (TABLE 2)[101]. Histological phenotypes of DILI are summarized in BOX 1. This demonstrates that a wide range of pathobiological processes are triggered by drugs and DILI may resemble a number of both acute and chronic liver diseases. In addition, adverse reactions from a single drug can present with different severities in different individuals, varying from asymptomatic liver biochemical test abnormalities to acute and subacute hepatic liver failure.

Although genome-wide association studies in DILI have indicated a major role for adaptive immunity in disease pathogenesis[102], the majority of DILI episodes do not demonstrate immunological features. Clinical features of immune-mediated or hypersensitivity drug reactions are not universally present but can be observed in one-quarter of patients, and include few or many of the following features: fever, cutaneous rash, facial periorbital oedema, lymphadenopathy, eosinophilia, lymphocytosis or the presence of reactive lymphocytes, and arthralgia[103]. For example, the anticonvulsants carbamazepine and phenytoin can cause liver injury that is most commonly associated with cutaneous hypersensitivity features[104], and liver injury caused by the antibiotic dapsone is associated with cutaneous hypersensitivity features in 90% of patients[105]. The skin rashes can vary from nonspecific morbiliform rashes to severe lesions such as erythema multiforme, drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome or Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN)[106,107].

Some other drugs such as α-methyldopa, nitrofurantoin and minocycline are associated with features that are indistinguishable from AIH, including the presence of anti-nuclear antibodies, hypergammaglobulinaemia and liver biopsy features compatible with AIH[108].

Autoimmune-like hepatitis associated with nitrofurantoin and minocycline are characterized by a prolonged latency to detection of >1 year[15].

A step-wise approach to clinical diagnosis. The majority of individuals with suspected DILI show increased levels of aminotransferase (such as aspartate aminotransferase (AST) or ALT) and/or alkaline phosphatase (ALP) above a certain threshold, sometimes accompanied by raised total bilirubin (TBIL) values detected during an investigation for nonspecific symptoms or during a diagnostic work-up for an acute viral hepatitis-like syndrome (BOX 2; FIG. 3). The latter does not usually point to a drug aetiology[101] unless associated skin or other systemic features are present that can reinforce the suspicion of drug toxicity[109]. Notably, the extent of increased liver enzymes alone is not sufficient to reflect the severity of DILI[110,111]; the development of ascites, coagulopathy and/or encephalopathy indicates severe disease[110]. Asymptomatic increases in transaminase levels that occur following exposure to a medication and that either resolve with continuation of the drug or following a decrease in dose

Table 1 | **Drug–patient interaction in DILI**

Drug factors	Patient factors	Effect on DILI risk
Drug exposure of hepatocytes		
• High daily recommended dose • Longer administration	• Bioavailability • Transporter activities • Drug-metabolizing enzyme activities	Increased exposure of hepatocytes to the drug increases the likelihood of inducing the toxic effects of the drug
Toxic effects on cellular homeostasis		
High potency of drug toxicity	• Cellular senescence • Impaired cellular adaptation	The toxic effect of the drug exceeding the patient's coping mechanisms leads to an increased likelihood of cellular dysfunction or death
Reactive metabolite formation	Increased activity of drug-metabolizing enzymes	Increased reactive metabolite formation
	Lysosomal dysfunction	Impaired functions to maintain cellular homeostasis
Mitochondrial toxicity	• Mitochondrial dysfunction • Older age	Enhanced mitochondrial damage
	Impaired mitophagy	Impaired functions to maintain mitochondrial homeostasis
Oxidative stress induction	Reduced antioxidants	Increased cellular damage due to oxidative stress
	• Female sex • Oestrogens (e.g., antioxidant effect)	Protective against cellular oxidative stress
BSEP inhibition	• Older age (e.g., age-related decline in cellular energy homeostasis) • Reduced activities of other bile acid transporters (such as MRP2, MRP3 and MRP4)	Enhanced bile acid accumulation in hepatocytes leads to cellular damage
Immune response, inflammation and tissue injury		
Anti-inflammatory drugs (such as NSAIDs), immunosuppressants (such as anti-TNF) and immunomodulatory drugs (such as antihistamines, statins and adrenergic antagonists)[1,196,197]	• HLA genotypes • PTPN22 (REF[189]) • Sex • Sex hormones (such as oestradiol, progesterone and testosterone receptors in immune cells) • Gut microbiota (discussed in REF.[1]) • X-linked immune-associated genes[198] • Immune senescence (such as ageing, premature senescence in HIV or chronic obstructive pulmonary disease)[199]	Intensified or dysregulated immune response augments inflammation and tissue injury
Tissue repair		
Drugs impairing tissue repair (e.g., histone acetylase inhibitors or sympathetic stimulants), drugs augmenting tissue repair (such as angiotensin-converting enzyme inhibitor/angiotensin II antagonists, statins and adrenergic blockers; reviewed in REFS[1,196,197])	• Older age • Premature senescence of hepatocytes • Altered FXR • Cirrhosis • Sex • Sex hormones (discussed in REF.[1]) • Telomere shortening (such in non-alcoholic fatty liver disease)[200]	Impaired tissue repair augments tissue damage, leading to a serious outcome

BSEP, bile salt export pump; DILI, drug-induced liver injury; FXR, farnesoid X receptor; MRP multidrug resistance-associated protein; PTPN22, protein tyrosine phosphatase non-receptor type 22; TNF, tumour necrosis factor.

Table 2 | **Case definitions and phenotypes of DILI**

Case definition	Drugs associated with phenotypes
Hepatocellular pattern of DILI	
ALT (or AST) alone is increased ≥5-fold above ULN or a ratio of ≥5	Acetaminophen, diclofenac, disulfiram, efavirenz, fenofibrate, isoniazid, lamotrigine, minocycline, nevirapine, nitrofurantoin, pyrazinamide, rifampicin and sulfonamide
Cholestatic pattern of DILI	
ALP alone is increased ≥2-fold above ULN or ratio ≤2	Amoxicillin–clavulanate, androgens, cephalosporins, chlorpromazine, erythromycin, flucloxacillin, oral contraceptives, penicillins, sulfonamide and terbinafine
Mixed pattern of DILI	
Ratio of >2 to <5	Carbamazepine, lamotrigine, phenytoin and sulfonamides
Autoimmune-like hepatitis	
Presenting features of acute or chronic DILI with serological and/or histological markers of idiopathic autoimmune hepatitis	Adalimumab, α-methyldopa, diclofenac, herbal supplements, infliximab, minocycline, nitrofurantoin and statins
Liver injury related to immune-checkpoint inhibitors	
Acute hepatitis can be severe; histological patterns include granulomas and central endotheliitis (caused by anti-CTLA-4 therapy) or lobular hepatitis (caused by anti-PD-1 or anti PD-L1 therapy)	Darvolumab[a], ipilimumab[b], nivolumab[a] and pembrolizumab[a]
Drug reaction with eosinophilia and systemic symptoms	
Drug-induced hypersensitivity reaction involving the skin with internal organ involvement	Allopurinol, carbamazepine, dapsone, lamotrigine, nevirapine, phenobarbitone, phenytoin and sulfonamide
Drug-associated fatty liver disease	
Non-alcoholic fatty liver disease attributable to exposure to specific medications	Amiodarone, 5-fluorouracil, irinotecan, methotrexate and tamoxifen
Acute fatty liver (microvesicular steatosis)	
Rapid liver involvement with extensive microvesicular steatosis	Amiodarone, didanosine and stavudine
Nodular regenerative hyperplasia	
Diffuse nodularity within the liver with wide and narrow sheets of hepatocytes at the centre and periphery, respectively, of nodules without advanced fibrosis leading to non-cirrhotic portal hypertension	Azathioprine, bleomycin, busulfan, oxaliplatin and 6-thioguanine
Vanishing bile duct (ductopenic) syndrome	
Cholestasis associated with gradual loss of intrahepatic bile ducts	Amoxicillin–clavulanate, azathioprine, carbamazepine, chlorpromazine, co-trimoxazole, erythromycin, flucloxacillin, phenytoin and terbinafine
Secondary sclerosing cholangitis	
Acute DILI with histological and/or features similar to those of primary sclerosing cholangitis on MRI	Amiodarone, amoxicillin–clavulanate, atorvastatin, infliximab, 6-mercaptopurine and venlafaxine
Peliosis hepatis	
Characterized by randomly distributed blood-filled cavities	Anabolic steroids, oral contraceptives and vitamin A
Hepatocellular adenoma or carcinoma	
Characteristics of hepatocellular adenoma or carcinoma based on imaging studies or histology	Contraceptive steroids, danazol and androgens

In most patients with acute drug-induced liver injury (DILI) in clinical practice, the DILI is characterized based on liver biochemistry as hepatocellular, cholestatic or mixed pattern. As the pattern of elevated liver enzymes can change over time[119], classification of DILI is based on the first set of laboratory tests available in relation to the clinical event[110]. Ratio (R value) of alanine aminotransferase (ALT) (or aspartate aminotransferase (AST)) activity expressed as fold elevation over its upper limit of normal (ULN) laboratory range to alkaline phosphatase (ALP) activity is used to define patterns of DILI. The pattern of liver injury has implications for prioritizing immediate investigations that are essential to exclude alternative causes of the injury as well as outcome. In patients with the hepatocellular pattern, DILI is more likely to resolve rapidly, but is associated with higher hazard ratio for fatality[150,154]. Other patterns of DILI should be characterized according to imaging or histological findings. CTLA-4, cytotoxic T lymphocyte antigen 4; DILI, drug-induced liver injury; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; ULN, upper limit of normal. [a]Anti-PD-1/anti-PD-L1. [b]Anti-CTLA-4.

Box 1 | **Histological phenotypes of DILI**

The histological phenotypes of drug-induced liver injury (DILI) can be stratified into 12 patterns. The most prevalent five phenotypes (acute and chronic hepatitis, acute and chronic cholestasis, and cholestatic hepatitis) accounted for 83% of 249 cases in a large series.[119] The correlation between histological findings and biochemical patterns is not perfect. Histological assessment is generally more accurate in assessing the extent and severity of damage than is reflected in biochemical testing[119]. Common drugs associated with histological phenotypes are listed below.

Acute hepatitis: fluoroquinolones, isoniazid, lamotrigine, α-methyldopa, minocycline, pyrazinamide and sulfonamides

Chronic hepatitis: carbamazepine, methyldopa, minocycline, nitrofurantoin, phenytoin and sulfonamides

Acute cholestasis: amoxicillin–clavulanate, chlorpromazine, erythromycin and flucloxacillin

Chronic cholestasis: anabolic steroids, cyclosporine and oestrogens

Cholestatic hepatitis: amoxicillin–clavulanate, azathioprine, carbamazepine, chlorpromazine, macrolides, sulfonamides and terbinafine

Granulomatous inflammation: allopurinol, carbamazepine, phenytoin and sulfonamides

Microvesicular steatosis: amiodarone, didanosine, stavudine, tetracycline and valproate

Macrovesicular steatosis: methotrexate and tamoxifen

Steatohepatitis: amiodarone, methotrexate and tamoxifen

Zonal necrosis: acetaminophen and halothane

Massive or submassive necrosis: isoniazid, phenytoin, pyrazinamide and sulfonamides

Nodular regenerative hyperplasia: azathioprine, bleomycin, busulfan and oxaliplatin and 6-thioguanine

are characteristic of anti-tuberculosis drugs or statins[112]. In most patients with suspected DILI, the DILI is classified as hepatocellular, cholestatic or mixed based on the results of liver biochemical tests (TABLE 2).

The first prerequisite for a diagnosis of DILI is a high degree of suspicion, so the physician should carefully enquire about exposure to prescription medication and over-the-counter drugs (such as acetaminophen), recording start and stop dates, as well as exposure to herbal and dietary supplements (which are often overlooked)[52]. Information on latency, course of reaction upon pharmacological therapy discontinuation, and time to resolution is needed to establish a compatible temporal relationship with the suspected causative agent. The time to onset of DILI varies considerably; most patients experience DILI within the first 3 months of therapy, although in some instances (such as amoxicillin–clavulanate-related DILI) symptoms can present with a considerable delay after treatment cessation[52].

The diagnosis of DILI currently relies on the exclusion of alternative causes (TABLE 3). This process encompasses a medical history to exclude alcohol abuse, sepsis and congestive heart failure, a search for recent episodes of syncope or hypotension (which would indicate ischaemic hepatitis), assessment of comorbidities and the individual's risk of acquisition of viral hepatitis and assessment of the local burden of infectious diseases that might involve the liver[52]. The pattern of injury provides guidance on additional investigations required. For example, a cholestatic anicteric pattern of injury requires the exclusion of primary biliary cholangitis and primary sclerosing cholangitis, whereas jaundice requires assessment for benign or malignant obstruction of the biliary tract. Liver imaging is routinely used in the evaluation of patients with liver injury, and all patients with suspected DILI should undergo abdominal ultrasonography to exclude biliary obstruction and focal lesions. In those with a cholestatic type of liver injury or in those with associated abdominal pain, additional imaging, such as magnetic resonance cholangiography or CT, might be required despite normal abdominal ultrasonography.

Screening for viral hepatitis A (detection of anti-hepatitis A virus IgM), B (detection of anti-hepatitis B virus core protein IgM or hepatitis B surface antigen) and C (detection of anti-hepatitis virus C antibodies) is mandatory in individuals with suspected DILI, except for those with a pure cholestatic pattern (TABLE 3). In addition, assessing for hepatitis C virus RNA (RNA-HCV), which has been found to be present in 1.3% of patients with initial suspicion of DILI[113], is also required. Hepatitis B virus DNA should be tested in hepatitis B virus surface antigen carriers to exclude chronic hepatitis B virus reactivation. Hepatitis E virus (HEV) is an emergent disease in western countries and is increasingly diagnosed in patients being evaluated for inclusion in DILI registries, in whom anti-HEV IgM seroprevalence ranges from 3% to 8%[114,115]. However, isolated detection of anti-HEV IgM is not reliable enough to diagnose HEV infection[116]. Accordingly, all patients with suspected DILI should be tested for HEV through detection of HEV RNA and anti-HEV IgM or IgG antibodies. Patients with a hepatocellular pattern of injury should also be assessed for AIH, including assessment for anti-nuclear autoantibodies and anti-smooth muscle autoantibodies and serum IgG levels. Nevertheless, the typical laboratory feature of AIH is a characteristic signature of several drugs including nitrofurantoin, minocycline, anti-TNF and statins, which makes the differential diagnosis between this particular phenotype of DILI and classic AIH a challenge[52]. Indeed, a liver biopsy — which is not generally required for the evaluation of a patient with suspected DILI — is justified when autoimmune features are present, as it can provide important diagnostic clues. For example, in a small study, hepatocellular cholestasis and portal neutrophils were indicative of DILI, whereas the presence of fibrosis suggested AIH[117]. In another study using immunohistochemistry of liver biopsies, portal infiltrates in DILI were formed predominantly by cytotoxic ($CD8^+$) T cells, whereas infiltrates in AIH had prominent mature B cells ($CD20^+$)[118]. Moreover, in contrast to patients with 'idiopathic' AIH, patients with DILI tend to show complete normalization (positive dechallenge) of serum aminotransferases over time.

In addition to its use for detecting AIH, liver biopsy can also be used in the assessment of patients with suspected DILI as incomplete normalization of liver biochemistry following drug discontinuation (negative dechallenge) raises the possibility of an alternative aetiology (such as veno-occlusive disease) or an atypical DILI phenotype. Liver biopsy can assist in these cases. Biopsy findings can also have prognostic value. In a systematic review of liver biopsies from 249 patients with DILI in a prospective observational cohort, higher degrees of necrosis, fibrosis stage, microvesicular steatosis and

ductular reaction were found to be indicative of a poorer prognosis, whereas eosinophils and granulomas were found more often in those with milder DILI[119]. Similarly, pathological assessment of patients with DILI who mainly presented with a cholestatic pattern showed that bile duct loss is predictive of the development of vanishing bile duct syndrome causing progressive cholestasis and leading to liver failure requiring transplantation or to death[120].

Serial aminotransferase measurements until complete normalization is also crucial for diagnostic reassurance in DILI. A steady decline in aminotransferase levels upon dechallenge supports the diagnosis, whereas worsening, persistence or incomplete resolution of laboratory abnormalities suggest a competing aetiology[52]. Nevertheless, clinicians should bear in mind that in a fraction of patients, DILI can evolve to ALF or become chronic despite stopping the drug, which is a further challenge in the diagnosis. Besides this, occasionally and upon careful questioning, the patient might recall similar symptoms after prior exposure to the agent and inadvertent drug rechallenge can be identified[121]. Overall, clinical symptoms can be informative in identifying drug signatures, establishing alternative causes and predicting outcome.

Causality assessment tools

A number of clinical scales to quantify the strength of association — the proof of causality, which is the Achilles' heel of adverse drugs reactions — have been proposed for DILI. Indeed, a valid structured and objective approach for identifying DILI is needed for research studies and to add consistency to clinical judgment by providing a framework that systematizes the features to be addressed in patients with suspected hepatotoxicity[122].

The general Naranjo Adverse Drug Reactions Probability Scale is a simple and easy to apply scale that is based on ten questions related to common evaluation criteria. However, this scale has demonstrated low sensitivity and reproducibility in a registry study owing to the presence of confusing questions and questions of no relevance to idiosyncratic DILI and is, therefore, not recommended for use[123]. Currently, the CIOMS/RUCAM scale is the only validated liver-specific scale used by regulators, the pharmaceutical industry and clinicians, and has been recommended by experts for causality assessment in DILI[110,124–126].

The CIOMS/RUCAM scale is composed of the following seven criteria: a temporal association between drug exposure and DILI recognition, rate of improvement with drug cessation, risk factors for DILI, exclusion of all other relevant causes of liver disorders, known drug hepatotoxic potential, recurrence of liver injury on drug re-exposure, and the potential influence of associated medications. This scale categorizes DILI as 'definite', 'highly probable', 'probable', 'possible', 'unlikely' or 'excluded'. Once a clinician has determined that liver injury could be drug-related, applying the CIOMS/RUCAM scale can further standardize and support the assessment. However, blind application of this scale is not a proof of causality and can lead to biased conclusions, particularly in poorly documented cases. Indeed, the CIOMS/RUCAM scale is mainly for supporting rather than excluding causality in DILI, and does not substitute for clinical judgment.

Despite the positive clinical uses of the CIOMS/RUCAM scale, it has several limitations. The scale is complex, includes ambiguous definitions, lacks data to support the selection and weighting of component domains, has a strong dependence on rechallenge data[122], and cannot obtain high categories of probability in some cases as dechallenge data are not included[125]. Patients with underlying liver disease can obtain lower scores owing to liver test fluctuations[15]. These shortcomings can explain the inter-observer variability and inconsistent test–retest reliability, even when this scale is used by expert raters[127]. In addition, the use of this scale is complicated in patients with DILI caused by herbal and dietary supplements, as there might be inaccuracies in the identification of the ingredients, pharmaceutical adulterants, chemical or botanical contaminations, lack of information on dose and duration of product consumption, and the potential for use of various complex formulations of plants or extracts. Differences in herbal terminology and limited product label information, if any, further contribute to the complexities in assigning causality in this context[18,128]. In addition, the CIOMS/RUCAM scale was developed in the early 1990s and, therefore, did not foresee the particular characteristics of new pharmacological agents for which new DILI mechanisms have been identified and for which DILI might present with a prolonged time to onset after drug withdrawal[129].

The DILIN uses a structured expert consensus opinion-based approach to assess causality, that has

Box 2 | **DILI criteria**

Clinical chemistry criteria

An international expert panel recommended that drug-induced liver injury (DILI) should be considered when any one of the following thresholds is met, even in the absence of symptoms:

- ALT or AST increase to ≥5-fold the ULN
- ALP increases to ≥2-fold the ULN
- TBIL concentration increases >2-fold the ULN associated with ALT or AST increases to ≥3-fold the ULN[110].

Hy's law, for the detection of DILI in clinical trials

- Key signals for potential DILI are imbalances in aminotransferase increases across treatment groups in relation to control groups and, as an indicator of more serious injury, the combination of aminotransferase and bilirubin increases fulfilling so-called Hy's law — which identifies individuals with hepatocellular jaundice — consisting of three components: aminotransferase increases to ≥3-fold the ULN more frequently than in (nonhepatotoxic) control or placebo groups.
- Individuals showing ALT or AST level >3-fold the ULN, combined with increases in serum TBIL to >2-fold the ULN, without initial findings of cholestasis, indicated by increased ALP.
- Absence of any alternative likely cause explaining the liver test abnormalities[157].

Hy's law is a reasonably sensitive and specific predictor of the potential of a drug to cause serious hepatotoxicity[148], indicating hepatocellular injury that is severe enough to impair hepatic function[157,193], and it is the US FDA's key marker to screen for the liver toxicity risk of a drug[149].

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ULN, upper limit of normal.

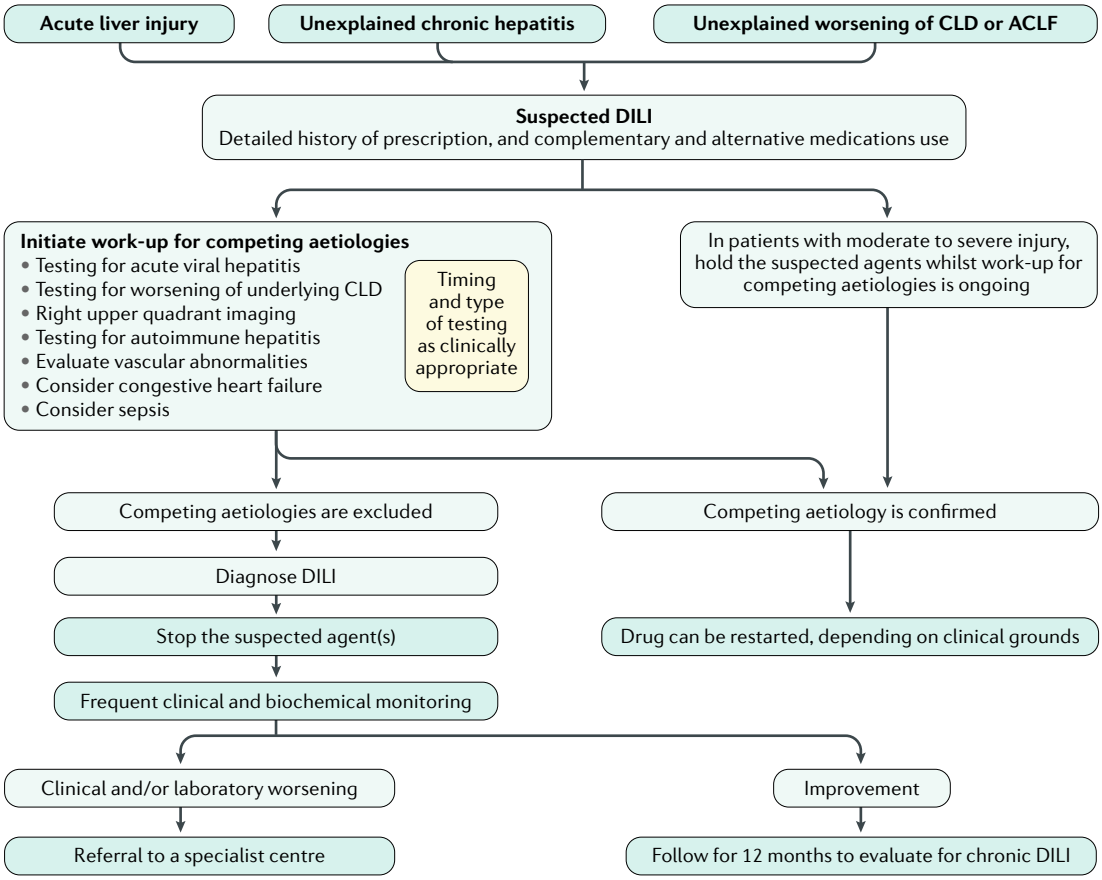

Fig. 3 | **Proposed algorithm to suspect, diagnose and manage idiosyncratic DILI.** Drug-induced liver injury (DILI) should be suspected in any individual presenting with acute liver injury, unexplained chronic hepatitis or unexplained worsening of chronic liver disease (CLD) or acute-on-chronic liver failure (ACLF). In such instances, a careful history of prescription, over-the-counter, and complementary and alternative medications should be obtained. In general, it is good practice to withdraw the suspected agent(s) while the work-up for competing aetiologies is undertaken. The work-up for competing aetiologies should be tailored according to the clinical presentation, but generally consists of testing for acute viral hepatitis, hepatobiliary imaging, and autoimmune serologies. If the competing aetiologies are excluded, the implicated drug should be permanently discontinued unless it is very important for clinical management. In patients with DILI and evidence of acute liver failure, prompt referral to a liver transplant centre should be considered. As some patients with DILI might develop chronic liver injury, it is important to follow up patients for 12 months to ascertain normalization of liver biochemistries and liver function.

shown higher agreement rates and likelihood scores than CIOMS/RUCAM, although the inter-observer variability is high with both instruments[130]. The scoring criteria of the DILIN instrument categorize DILI likelihood as 'definite', 'very likely', 'probable', 'possible' or 'unlikely'[130,131]. The lack of reproducibility with this instrument might be due to the absence of numerical scores for each of the items evaluated. Indeed, opinions between evaluators are highly dependent on the examiner's prior knowledge or information provided. In addition, expert opinion can weight the assessment of clinical signatures for DILI that are characteristic of specific drugs. Nonetheless, the reliability of this instrument in daily clinical practice is unknown[132].

Another important limitation of the CIOMS/RUCAM scale is that it cannot discriminate between concomitant hepatotoxic drugs with the same temporal sequence. In an attempt to try to circumvent this limitation, the liver-specific Digestive Disease Week Japan (DDW-J) scale, which was modified from the CIOMS/RUCAM scale, includes an in vitro drug lymphocyte transformation test (LTT), which assesses whether the DILI reaction is mediated by a T cell response against the drug in its evaluation criteria[133]. However, the lack of standardization among laboratories of the LTT has prevented its generalization. Indeed, a modified LTT measuring granzyme B and cytokine production could not reliably establish causality[134]. In a further attempt to improve diagnostic capability, a hepatotoxicity assay using monocyte-derived hepatocyte-like cells from patients with idiosyncratic acute liver injury has been developed, and has shown promising results. This in vitro test awaits external validation, and takes several weeks to carry out, reducing its potential use in the clinic[135]. An updated CIOMS/RUCAM scale, which incorporates an expanded list of alternative causes to be excluded and a new definition of rechallenge, has been proposed, but its performance needs to be tested in large cohorts of patients with well-characterized DILI[136]. A collaborative international working group led by DILIN has been set up to develop

Table 3 | **Laboratory, imaging and histological assessment in DILI diagnosis**

Assessment	Diagnostic value
Liver Tests	
Elevated aminotransferases (ALT and AST)	• Indicate hepatocellular damage • Substantially increased values suggest hypoxic damage to the liver
Elevated creatine kinase	In association with elevated AST that is increased more than ALT, indicates muscle injury rather than liver damage
Elevated total bilirubin	• Useful to detect impaired hepatic uptake, conjugation or excretion; biliary obstruction; and/or haemolysis • Isolated elevation even of the conjugated fraction does not mean DILI • Of diagnostic and prognostic value when associated with an increase in ALT (Hy's law)
High ALP	Cholestasis if bone disease can be excluded; also elevated in biliary obstruction and infiltrative diseases
Elevated γ-glutamyl transferase	Indicates cholestasis when associated with an increase in ALP, isolated elevation is not indicative of liver injury. Concomitant elevation of mean corpuscular volume suggests alcoholic liver disease
Low albumin, high INR	• Can indicate impaired hepatocellular function • Is altered in cirrhosis of any cause
Laboratory work-up	
Serology for hepatitis A, B, C and E virus	Can detect viral hepatitis
Serology for CMV, HSV and EBV	• Should be carried out in those with systemic symptoms • Includes anti-CMV IgM and IgG, anti-HSV IgM and IgG and anti-EBV IgM and IgG
Anti-nuclear and anti-smooth muscle IgG	Autoimmune hepatitis (can be drug-induced)
Ceruloplasmin levels, transferring saturation and α1-antitrypsin levels	Wilson disease, haemochromatosis (in anicteric hepatocellular damage) and α1-antitrypsin deficiency, respectively
Imaging and histology	
Ultrasonography	• Normal in DILI • Is mandatory to exclude focal lesions and biliary tract disease • No additional imaging techniques are required in individuals with 'viral hepatitis-like' syndrome
MRI	• Necessary in those with suspected cholestasis and/or accompanying abdominal pain • Biliary tract disease (benign or malignant) might require endoscopic retrograde cholangiopancreatography in addition to MRI • Can also help to exclude non-alcoholic fatty liver disease, focal lesions or ischaemic injury
Liver biopsy	Can be useful in those with: • Autoimmune hepatitis phenotype • Liver injury related to immune-checkpoint inhibitors • Suspected atypical DILI presentations (such as sinusoidal obstruction syndrome, peliosis hepatis or microvesicular steatosis) • Negative or incomplete dechallenge (for assessing severity and/or competing aetiologies)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMA, cytomegalovirus; DILI, drug-induced liver injury; EBV, Epstein–Barr virus; HSV, herpes simplex virus; Ig, immunoglobulin; INR, international normalized ratio; TNF, tumour necrosis factor.

an objective, online computer program with a simplified scoring system, evidence-based criteria and refined weighting for wider applicability in the clinical setting.

New biomarkers

The shortcomings of the traditional DILI biomarkers in terms of liver specificity, prediction of DILI outcome and mechanistic insights has led to international collaborative efforts to identify and validate new biomarkers[137] (FIG. 4).

Both microRNA-122 (miR-122) and glutamate dehydrogenase (GLDH) have been supported by the FDA for further exploration as liver-specific biomarkers in the clinic[138]. Approximately 70% of the miRNA content in the liver is miR-122 (REF.[139]). Although miR-122 levels are more specific for liver injury than ALT or AST levels, substantial inter-individual and intra-individual variability has been reported in circulating levels in healthy adults[140]. This variation might be due to the release of miR-122 from healthy hepatocytes, which can influence physiology in remote tissues[141,142]; however, the relevance of this variation for using miRNA as a biomarker for DILI is not clear, as relevant studies have not used similar methods[143]. GLDH is a mitochondrial protein[144]. In a large study of healthy volunteers[140], GLDH had a lower inter-individual and intra-individual variation than miR-122, and is released into the circulation during

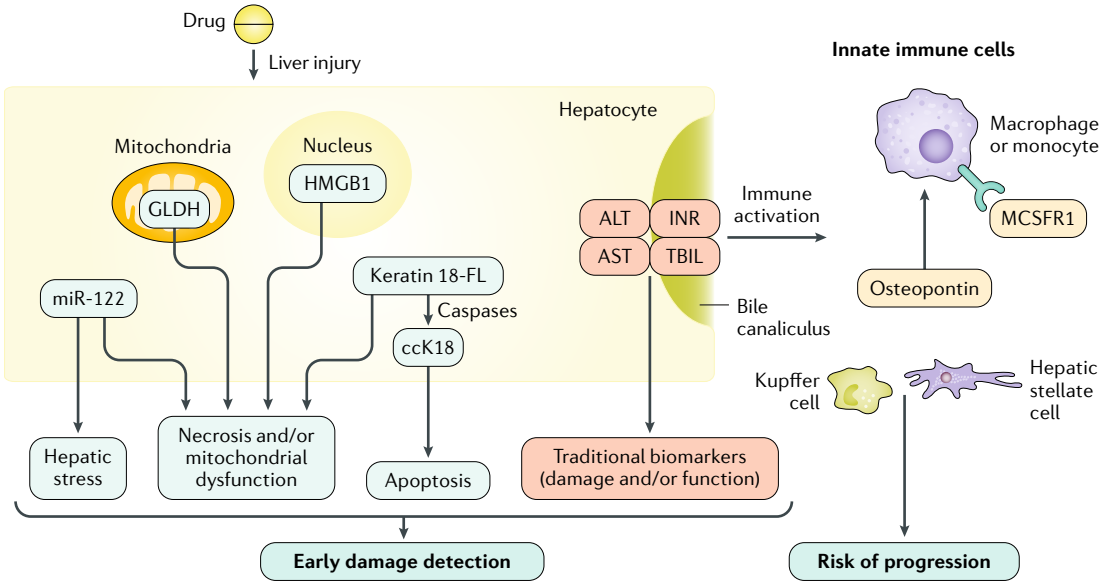

Fig. 4 | **Traditional and investigational biomarkers of DILI.** One active area of research is the identification of biomarkers that could detect the initiation of each of the pathophysiological steps of DILI, in view of the poor specificity of the traditional biomarkers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin levels (TBIL) and international normalized ratio (INR). During hepatocyte necrosis, microRNA (miR)-122, glutamate dehydrogenase (GLDH) and full-length cytokeratin 18 (keratin 18-FL) are released. Accordingly, GLDH levels have been proposed as an approach to the identification of mitochondrial toxicity as a mechanism of DILI[194]. In addition, the serum ratio of caspase-cleaved cytokeratin 18 to full-length cytokeratin 18 (ccK18/K18) has been proposed as an estimate of the ratio of apoptosis to necrosis during DILI. High mobility group box 1 protein (HMGB1), miR-122 and DNA are among the multiple damage-associated molecular patterns (DAMPs) that are released from dysfunctional hepatocytes and activate innate immune cells, which in turn release macrophage colony-stimulating factor receptor 1 (MCSFR1). Osteopontin is involved in the migration and infiltration of inflammatory cells and seems to promote hepatocyte regeneration. Identifying biomarkers of innate immune cell activation in the liver is ongoing. Acetylated HMGB1 has been proposed as a biomarker to address this, but the integrity of at least one of the key studies has been questioned[195].

necrosis or mitochondrial dysfunction by hepatocytes, supporting its role as a biomarker for DILI. Other tentative biomarkers for DILI include high mobility group protein B1 (HMGB1) and the ratio of cleaved cytokeratin 18 to full-length cytokeratin (FIG. 4). HMGB1 is a nuclear protein that is released during necrosis of most cell types and can act as a DAMP to activate innate immune cells.

Other potential biomarkers for DILI include markers of the immune response. Macrophage colony-stimulating factor receptor (MCSFR) is found on macrophages and monocytes as the receptor for colony-stimulating factor, a cytokine that controls the proliferation, differentiation and function of macrophages. The levels of MCSFR in blood could reflect activation of innate immune cells (such as inflammation), although the specificity of this marker for idiosyncratic DILI remains to be established in the ongoing biomarker consortium TransBioLine, funded by the Innovative Medicines Initiative of the European Union. In addition, osteopontin, which has a role in the migration and infiltration of inflammatory cells into the liver, is considered a candidate biomarker for DILI.

In one international collaboration, biomarkers were quantified in serum samples collected from patients with DILI within 2 weeks of DILI onset[140]. Although the international normalized ratio (INR, a measure of the ability of blood to clot) was the best single biomarker for predicting which patients with DILI would progress to liver failure, osteopontin had the best performance of the candidate biomarkers in predicting liver failure, exceeding that of the traditional liver safety biomarkers, including TBIL. This study also addressed whether adding any of the newer biomarkers would improve the predictive ability of the Model of End-stage Liver Disease (MELD), which is based on traditional blood biomarkers and is used to prioritize patients for liver transplantation. In this study, incorporating total keratin 18 (K18) and MCSFR levels[140] improved prediction of which patients with DILI would progress to liver failure. In addition, serum levels of miR-122 have been suggested to predict liver failure outcome from DILI[145], although these findings require further validation. Finally, low blood levels of some cytokines (along with albumin) were predictive of death within 6 months of hepatotoxicity onset[146].

Prognosis

The prognosis of patients with DILI is related to many different factors. Patients diagnosed in population-based studies[13,32] generally have a more favourable prognosis than patients recruited in tertiary referral centres[15]. In population-based cohorts, only ~30% of patients with DILI present with jaundice, whereas this feature is present in 60–70% of patients seen in tertiary referral centres[15,16].

The so-called 'Hy's law', named after the late Hyman Zimmerman, is still widely used to predict outcomes in patients with DILI[111] (BOX 2). Hy's law was based on the observation that, in patients with isoniazid-induced hepatocellular jaundice[147], the fatality rate from liver failure or the need for liver transplantation is ≥10%[147]. A fatality rate of 10% has subsequently been observed for many other drugs and is now used by the FDA to predict the risk of hepatotoxicity of drugs[148,149]. If more than one patient meets the criteria for Hy's law in a clinical trial, the implicated drug is unlikely to be marketed as it is likely to have a post-marketing hepatotoxicity problem[147–149].

The validity of Hy's law has been confirmed in several studies[16,33,113]. Patients with hepatocellular jaundice (fulfilling Hy's law) had the worst prognosis in two studies, with a fatality rate of 7–13%[16,33], whereas patients with cholestatic jaundice had the highest fatality rate in the first report from the DILIN cohort of 14%[113]. This value was higher than that in the Swedish and the Spanish DILI cohorts, which had fatality rates of ~5–8%[16,33]. However, jaundice induced by different drugs can have different prognoses. For example, in one study of patients with jaundice due to idiosyncratic DILI, the mortality rate varied from 40% for halothane to 0% for erythromycin[33]. Researchers from the Spanish hepatotoxicity network have tried to optimize the definition of Hy's law and develop a model for predicting ALF in patients with DILI[150]. These researchers have developed a prognostic algorithm that has been found to be more reliable than Hy's law, in particular in identifying patients who will not develop ALF[150].

Other biochemical, histological and clinical features can also affect prognosis in patients with DILI. Indeed, the occurrence of peripheral and hepatic eosinophilia in patients with DILI is associated with a favourable prognosis in patients with disulfiram-induced liver injury[151] and in patients with liver injury from many other drugs with well-documented hepatotoxicity[104,152]. Although SJS/TEN rarely accompany DILI, when they do they are associated with a high fatality rate, particularly in individuals with jaundice[109]. In patients with SJS/TEN, mortality is higher in those with severe hepatic dysfunction[109], although it is unclear whether this is due to the effects of the idiosyncratic drug reaction in the liver or if those more severely affected by SJS/TEN develop liver dysfunction secondary to sepsis.

The majority of patients with DILI recover completely, and only a small minority experience chronic DILI, which is defined as the persistence of liver biochemical or imaging abnormalities after 1 year. Only 8% of 292 patients in a prospective Spanish DILI registry developed chronic DILI, including liver cirrhosis and ductal lesions, with no pattern of DILI associated with progression to chronic DILI[153]. Old age, dyslipidaemia and the severity of the acute episode were risk factors for chronic DILI. Anti-infective drugs and statins were implicated in 50% of patients[153]. In another large cohort study, ~10% of 1,089 patients with DILI died within 2 years; of those in whom DILI was the primary cause of death, 74% had acute, 13% chronic, 7% acute on chronic, and 6% acute cholestatic failure[154].

Clinical trials and post-marketing

DILI is one of the major reasons for late-stage attrition in drug development[2,155,156], and non-negligible safety risks during clinical trials. Careful patient selection, thorough monitoring of clinical symptoms and standard liver chemistries, defined rules for stopping drug administration and systematic signal detection and assessment remain the core elements of DILI risk management.

The FDA industry guidance document 'Drug-Induced Liver Injury: Premarketing Clinical Evaluation'[157] laid the foundation for the systematic and standardized diagnosis, assessment and management of DILI in clinical studies. To minimize the risks of DILI during early phase clinical trials, in line with the FDA DILI guidance, healthy individuals and patients are usually included only if liver chemistry findings are normal at baseline. However, for later stage trials, once the initial assessment of the safety profile of the drug is considered satisfactory, the inclusion of patients with mild underlying liver abnormalities is encouraged by the FDA to better reflect real-life conditions expected after marketing of the drug.

Owing to the absence of more advanced, fully qualified, sensitive and specific biomarkers for DILI, monitoring of liver safety relies on the standard battery of liver chemistry tests: ALT, AST, ALP and TBIL (BOX 2)[158,159]. Monitoring intervals are adapted to the development stage, and preferably to the number of patients exposed to the drug and previously observed liver safety profiles. Typically, liver chemistry is measured twice weekly during phase I studies, and down to once per month during later stage trials, provided no liver safety signal has been observed in earlier studies[160].

If liver chemistry abnormalities suggest DILI during clinical trials, treatment interruption is the most important measure to avoid progression to more serious injury[157,161]. The FDA DILI guidance offers a set of rules for determining when administration of a drug that is suspected to have caused acute liver injury should be stopped[157], the first of which recommends discontinuing the drug if ALT or AST levels exceed eight times the upper limits of normal (ULNs) on treatment. However, in practice, drug administration is mostly stopped at lower increases in aminotransferase levels to minimize risk[68,162]. Although this conservative approach is taken in the presumed interest of patient safety, premature treatment stoppage diminishes the opportunity to see adaptation to effects on the liver, if this occurs[2,163]. Provided close patient observation is ensured, untimely discontinuation of drug administration should be avoided to minimize signals falsely suggesting serious toxicity[2,157,163]. For signal assessment, in addition to standard statistical analysis, a systematic workflow using data visualization that is based and expanding upon the Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) process of the FDA[164,165], an interactive visual approach to the assessment of hepatotoxicity potential, has been suggested to optimize the use of available data and to support proper interpretation of the liver safety profile of a drug[166].

For drugs that have received regulatory approval despite a pre-marketing signal for potential liver toxicity, regulators will mandate the inclusion of respective

safety information and risk mitigation measures in the product label. Depending on the severity of the signal, this information might include the mention of hepatotoxicity in the adverse reactions section, in the warnings and precautions section, or even in a dedicated box warning section, along with stipulation of monitoring intervals for liver chemistry tests. A key problem in the post-marketing setting is that monitoring intervals specified on the label are not always strictly followed, potentially increasing the risk of liver toxicity[167–169]. If liver safety of a new drug cannot be fully established in pre-marketing trials, further studies might be required after regulatory approval of the drug to assess potential hepatotoxicity (BOX 3).

Management

In many patients, DILI can spontaneously improve without the need for active treatment. The key steps in the management of DILI are timely recognition and withdrawal of the offending medication(s), timely referral of individuals with drug-induced ALF to a liver transplantation centre and pharmacotherapy (FIG. 3). A delay in timely identification and immediate withdrawal of isoniazid and other anti-tuberculosis medications is one of the risk factors for a worse outcome, such as liver transplantation or death[10]. Rechallenge with a suspected agent is strongly discouraged unless it is clinically imperative; in such instances, frequent biochemical monitoring is advised[170]

Pharmacological therapy

Therapeutic options for hepatocellular DILI are limited. Corticosteroids are frequently administered to patients with significant DILI (such as that associated with liver dysfunction) in an empirical manner, but there is no evidence to support their use except in patients in whom acute AIH cannot be excluded or to treat hepatotoxicity due to immune-checkpoint inhibitors (ICIs). Currently, the mainstay in the treatment of hepatotoxicity due to ICIs is prednisone, with an additional or alternative immunosuppressant such as mycophenolate mofetil[171], although the evidence to support this therapy is inconclusive at best. In one study[172] in patients who had at least grade 3 hepatotoxicity according to Common Terminology Criteria for Adverse Events, version 4.03 (that is, ALT ≥5-fold the ULN) from ICIs, corticosteroid administration to 67 patients led to a response in all but three. However, the decision to start corticosteroid therapy in this population remains controversial. In another study, the management of patients with ICI-induced liver injury was tailored according to biochemical (TBIL >2.5 mg per dl and/or INR >1.5) and histological markers of severity. Using these pre-established guidelines, 6 of 16 patients (38%) with ICI-induced liver damage who did not receive corticosteroids showed spontaneous improvement[173]. In another cohort of 128 patients with melanoma treated with ICIs, five of ten with DILI received steroids, but DILI resolved in all patients in a median time of 4.7 weeks in those receiving no steroids, compared with 8.6 weeks in those who received corticosteroids[174]. A suggested algorithm to detect and manage hepatotoxicity due to ICIs in patients with cancer who are considered for ICI therapy in accordance with current practice is shown in FIG. 5.

Cholestyramine, a bile acid resin, can be administered to patients with acute liver injury caused by leflunomide, an immunomodulatory agent used for the treatment of rheumatic arthritis and psoriatic arthritis, to accelerate elimination of this drug[10]. *N*-Acetylcysteine (NAC), an antidote agent for acetaminophen toxicity, was investigated in a randomized placebo-controlled trial for non-acetaminophen-induced ALF that included DILI as one of the subgroups[175]. In this study, the transplant-free survival of individuals with non-acetaminophen-induced ALF who received NAC was significantly higher than those who did not receive NAC (58% versus 27%, $P < 0.05$). Individuals with cholestatic DILI with severe itching might benefit from treatment with an antihistamine (such as diphenhydramine or hydroxyzine) or a bile acid resin (such as cholestyramine). It is not uncommon for clinicians to try ursodeoxycholic acid in individuals with significant cholestatic DILI; indeed, 30% of patients in the DILIN prospective study were given ursodeoxycholic acid[170], but there are no data to support its use in this indication.

Liver transplantation

Although there are no strict criteria regarding when to refer patients with DILI for liver transplantation, a general rule is when ALF develops as evidenced by coagulopathy, when early mental status changes or when renal dysfunction occurs. In individuals with hepatocellular DILI, progressive worsening of jaundice should also prompt the clinician to consider initiating a referral to a nearby liver transplant centre.

Quality of life

After experiencing a severe adverse drug reaction, many patients develop fear and anxiety towards medications[176] including worries about recurrence, re-exposure to the drug, effect on their fertility, or developing adverse drug reactions to other drugs. Such a negative perception of medications can adversely affect their QOL and can

Box 3 | **Post-marketing pharmacovigilance**

As drug-induced liver injury (DILI), in particular idiosyncratic forms, is a rare yet serious adverse drug reaction, the likelihood of detecting a robust signal before marketing authorization, even given increasingly large trials in drug development programmes, is low. Thus, in the absence of cases clearly fulfilling Hy's law, there is a genuine risk that a signal is observed only after launch of a drug[157,164], during post-marketing surveillance studies, from specific DILI registries or from spontaneous reporting. Although dedicated post-marketing surveillance studies and registries help generate high-quality data and structured output, unsolicited spontaneous reports often lack adequate quality and completeness to support timely post-marketing detection and causality assessment of suspected DILI. In addition to a widespread lack of awareness of DILI in clinical practice, other challenges include the following:

- Missing baseline liver chemistry values
- Lack of adherence to recommended monitoring intervals, even with products that carry a box warning for DILI[167–169]
- Treatment with multiple drugs, including self-medication with, for example, herbal remedies and dietary supplements

To address these challenges and overcome respective deficiencies, more in-depth training on the background, detection and management of DILI for physicians in hospital and clinical practice would be helpful.

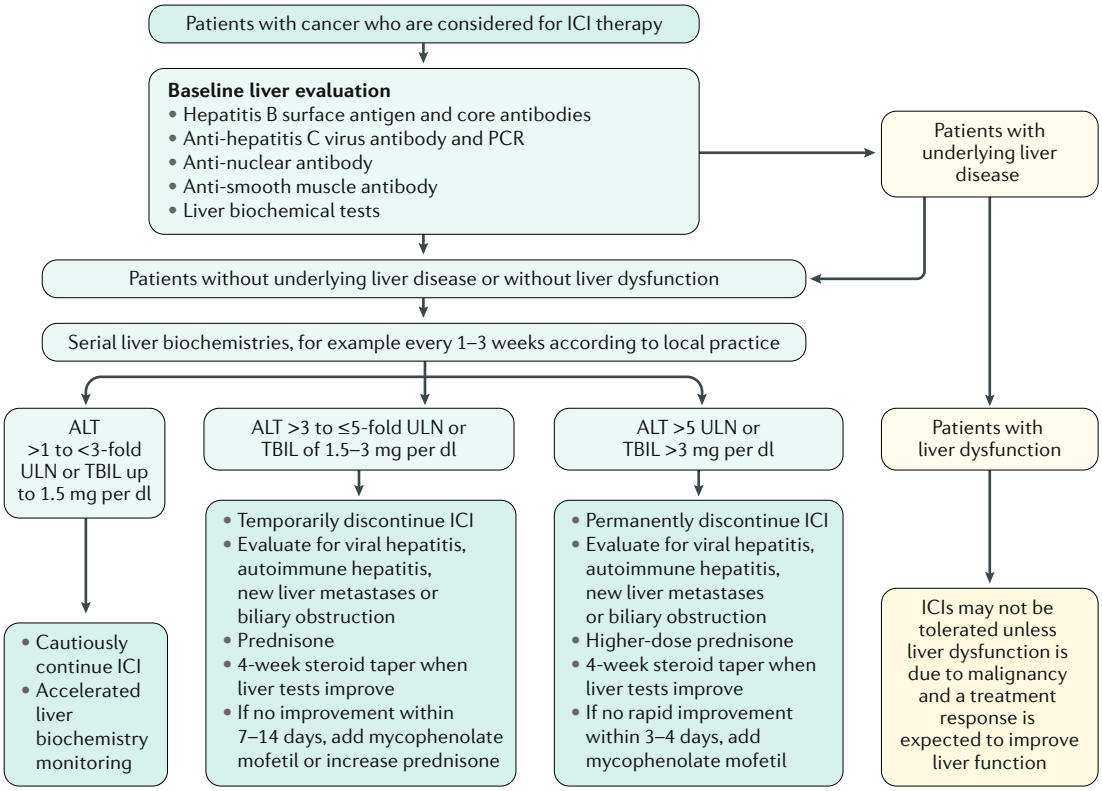

Fig. 5 | **Proposed detection and management of hepatotoxicity due to ICIs in patients with cancer.** Patients with malignancies who are considered for immune-checkpoint inhibitor (ICI) therapy should undergo a baseline evaluation of liver biochemistries and liver function tests, viral hepatitis serology and autoimmune markers. ICI therapy might not be suitable in those with underlying liver dysfunction unless the underlying liver dysfunction is related to the malignancy. In patients without serious underlying liver disease, ICI therapy can be initiated with serial liver biochemistry monitoring every 1–3 weeks, depending on local practice. The emergence of elevated liver biochemistries should lead to management according to their levels. In patients with alanine aminotransferase (ALT) levels >1 but ≤3-fold the upper limit of normal (ULN) or total bilirubin (TBIL) elevation up to 1.5 mg per dl, ICIs can be continued but the patients should be considered for accelerated liver biochemistry monitoring. In patients with incident ALT levels >3 to ≤5-fold the ULN or TBIL 1.5–3 mg per dl, temporary discontinuation of the ICIs whilst initiating a work-up for competing aetiologies should be considered, together with considering initiation of therapy with prednisone. If there is not a rapid response, additional immunosuppressive therapy with mycophenolate mofetil or an increased prednisone dose should be considered. In patients who develop ALT >5-fold ULN or TBIL >3 mg per dl, ICIs should be permanently discontinued and therapy with prednisone should be initiated.

affect treatment adherence, and might increase the likelihood of discontinuation of needed therapy[177]. The most widely accepted questionnaire to measure QOL is the short-form-36 (SF-36)[178], a standardized tool that is used to assess patient health across eight dimensions. An alternative method is the Beliefs about Medicine Questionnaire (BMQ)[179].

As observed in cutaneous adverse drug reactions[176], patients who have had DILI could develop fear, anxiety, disbelief towards medicines and discomfort, all of which can lead to deterioration in their QOL. Indeed, one study in South Korea demonstrated higher indexes of anxiety and depression in patients with DILI induced by herbal and dietary supplements than in healthy individuals and in patients with DILI with other aetiologies[180]. Interestingly, the DILIN group reported that patients with persistent liver enzyme elevation 12 months after DILI onset had significantly poorer SF-36 physical summary scores at DILI onset and throughout follow-up than those in whom liver enzyme levels resolved[181].

Acetaminophen overdose is the most common cause of drug-induced ALF in the USA[182]. Whereas only 25% of patients with idiosyncratic drug-induced ALF have spontaneous recovery, the rate is >65% in patients with acetaminophen-induced ALF[182]. Despite the better short-term orthotopic liver transplantation (OLT)-free survival in patients with acetaminophen-induced ALF, patients with spontaneous recovery after acetaminophen-induced ALF have lower scores for general health, a longer duration of impaired mental and physical health, and a longer duration of activity limitations due to poor health, pain, depression and anxiety than those with spontaneous recovery from non-acetaminophen ALF and OLT (of different aetiologies including idiosyncratic DILI)[183]. However, this finding could be explained by the fact that survivors of acetaminophen-induced ALF have significantly higher rates of psychiatric and substance abuse disorders[183].

Taken together, although the evidence is limited, patients seem to have poor physical and psychological

status and low QOL after certain types of DILI presentation, such as persistent liver enzyme elevation and ALF with or without OLT. Otherwise, we lack studies of QOL in those with specific DILI phenotypes, and studies assessing the beliefs, attitudes and expectations after an episode of hepatotoxicity for both patients and physicians. Indeed, the effect of idiosyncratic DILI on QOL remains a neglected area of research and requires further study. Interestingly, a survey in 2014 found that primary care physicians express several liver safety concerns regarding prescription of statins despite their safety and efficacy, leading to their underutilization[184]. An integrative model that includes diverse phenotypic expressions, psychological attitudes and outcomes imposed by DILI and its effect on patient health should encourage the evaluation of QOL. Thus, it would be essential to conduct a QOL survey with each patient during and after the acute phase of a DILI episode.

Outlook

The prediction of DILI risk with preclinical cell-based and organelle-based assays and the chemical properties of drugs promises to enable selection of the most favourable characteristics among a group of compounds to advance to in vivo testing in drug development. Several issues need to be further investigated in the future, such as how the identification of drug-induced cellular alterations are involved in the pathogenesis of idiosyncratic DILI, and if these stressors are necessary for idiosyncratic DILI development or if they are surrogates for hepatic exposure to and metabolism of lipophilic drugs. In addition, whether the fitness of adaptive responses to these stressors, mitochondrial quality control, antioxidant defence, induction of alternative routes of transport or detoxification of bile acids or drug metabolites dampens the progression from minimal to severe liver injury remains to be established (for example, the unfolded protein response in the ER or the mitochondria could generate organelle-specific protective responses). Furthermore, elucidation of the role of immune tolerance as a mechanism of adaptation to reduce DILI progression could potentially lead to novel approaches to the prevention of severe liver injury. Recent attempts to integrate mechanisms and patient risk factors using quantitative systems toxicology modelling are showing promise towards predicting DILI risk[185].

Another important area for research is the suppression of cholestasis and cell death, as well as innate immune responses, as approaches to the treatment of established acute liver injury. Thus, the role of various cell death pathways and cholestatic injury mechanisms in DILI needs to be identified to exploit new therapies to suppress overt liver injury as it reaches certain thresholds that predict progression of the injury, perhaps informed by early identification of predictive biomarkers.

It is unrealistic to expect drugs to be free from adverse effects; thus, discovery and development of diagnostic, prognostic and mechanistic biomarkers that enhance the safe use of drugs are integral for precision medicine. In addition to blood-based biomarkers (see Diagnosis, screening and prevention, above), technologies such as mass cytometry, single cell genetics and next-generation sequencing would permit in-depth immunophenotyping of circulating and infiltrating immune cells as well as microRNA profiling to potentially identify changes that are unique to DILI. With discovery science informed by recent advances in the understanding of the pathogenesis, the use of advanced analytical methods and tools such as deep machine learning would bring about a step change in the application of a combination of biomarkers in an individual clinical scenario to support decision-making.

Genome-wide association studies led by international consortia have identified a number of genetic risk factors for DILI. HLA genotypes and haplotypes have been associated with hepatic adverse reactions to >20 drugs. HLA genotyping is widely accessible and affordable, and can assist diagnosis in selected clinical scenarios[186]. Indeed, high negative predictive values (>95%) of these alleles can be used to rule out a particular drug as a causative agent when the pre-test probability of DILI is low and an alternative competing diagnosis exists. In addition, carriage of a specific HLA allele favours attribution of liver injury to a particular drug when, because of exposure to a combination of drugs, definite conclusions cannot be drawn. HLA typing could be an adjunct in the differential diagnosis of DILI versus AIH, as with international AIH diagnostic criteria which attribute additional scores for carriage of HLA-DRB1*0301 and DRB1*0401 (REF.[187]). The performance characteristics of HLA alleles used as a test in patients with DILI are comparable to those of autoantibodies and the immunoglobulin profile that are performed routinely in the investigation of acute liver injury[102].

In addition, HLA alleles that are associated with a variety of adverse reactions including DILI, cutaneous hypersensitivity and drug-induced pancreatitis have substantial overlap. Thus, one potential consideration is to treat all relevant HLA genotypes as one panel covering different forms of adverse drug reactions such as cutaneous hypersensitivity (related to carbamazepine, abacavir and dapsone) and drug-induced pancreatitis (attributed to thiopurine immunosuppressants) in addition to DILI, thereby improving their clinical application[186]. More recently, genome-wide association studies have revealed non-HLA genetic variants that are associated with DILI secondary to the therapeutic use of interferon-β[188] as well as DILI in general[189]. In addition, ~30–40% of functional variability in pharmacogenes is estimated to be attributed to rare variants requiring sequencing-based approaches for discovery[190].

As with most polygenic disorders, genetic tests have not been used so far to risk-stratify individuals prior to drug prescription with the intention to prevent DILI. With the aim of introducing polygenic risk prediction into clinical care, investigators recently developed and validated genome-wide polygenic scores for five common diseases[191]. Truly individualized medicine would be available when a similar polygenic score related to adverse drug reactions is developed ready for clinical application.

From a therapeutic standpoint, idiosyncratic DILI is still an orphan disease. This is the consequence of a number of factors. First, the incomplete understanding of DILI pathogenesis and the complexity of its underlying

mechanisms have hampered efforts to develop animal models relevant to human idiosyncratic DILI. Despite the efforts to establish a better approach to human DILI, such as inhibiting normally tolerogenic immune pathways to render mice susceptible to DILI[192], there is no widely accepted animal model and none of the existing in vitro and in silico models of hepatotoxicity are approved by the regulatory agencies for preclinical drug development. On the other hand, the discovery of mechanistic biomarkers and genetic information brings hope for improving the detection of DILI in clinical trials. The current absence of diagnostic DILI biomarkers impairs the accurate DILI case qualification process, which is crucial to the correct enrolment of patients in trials to assess older or new molecules in the treatment of this condition. Presumably, international efforts already in place (Translational Safety Biomarker Pipeline, TransBioLine) to further discover and validate specific DILI biomarkers will change the landscape over the next years. Finally, the relative rarity of the disease along with the myriad of phenotypic presentations, which further reduce the potential randomization of eligible patients, precludes the undertaking of clinical trials with adequate statistical power. Nevertheless, the potential benefit from older agents used empirically in DILI, such as ursodeoxycholic acid and steroids, is worthy of evaluation in well-designed clinical trials. This is now feasible by taking advantage of international consortia that prospectively recruit patients with DILI. Indeed, prospective DILI registries will remain an invaluable resource for testing diagnostic biomarkers and promoting new therapeutic strategies in the near future.

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1. Chen, M., Suzuki, A., Borlak, J., Andrade, R. J. & Lucena, M. I. Drug-induced liver injury: interactions between drug properties and host factors. *J. Hepatol.* **63**, 503–514 (2015).
A concept paper addressing drug properties, patient factors and their interplay in DILI.
2. Kaplowitz, N. Idiosyncratic drug hepatotoxicity. *Nat. Rev. Drug Discov.* **4**, 489–499 (2005).
Key review highlighting important issues and potential mechanism underlying idiosyncratic DILI.
3. Reuben, A. et al. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. *Ann. Intern. Med.* **164**, 724–732 (2016).
4. Donnelly, M. C. et al. Acute liver failure in Scotland: changes in aetiology and outcomes over time (the Scottish Look-Back Study). *Aliment. Pharmacol. Ther.* **45**, 833–843 (2017).
5. Zimmerman, H. J. & Maddrey, W. C. Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: analysis of instances of therapeutic misadventure. *Hepatology* **22**, 767–773 (1995).
6. Watkins, P. B. et al. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily. *JAMA* **296**, 87–93 (2006).
7. Chen, M., Borlak, J. & Tong, W. High lipophilicity and high daily dose of oral medications are associated with significant risk for drug-induced liver injury. *Hepatology* **58**, 388–396 (2013).
8. Stevens, J. L. & Baker, T. K. The future of drug safety testing: expanding the view and narrowing the focus. *Drug Discov. Today* **14**, 162–167 (2009).
9. Avigan, M. I. & Muñoz, M. A. Perspectives on the regulatory and clinical science of drug-induced liver injury (DILI). en *Methods in Pharmacology and Toxicology* (eds. Minjun Chen & Yvonne Will) 367-393 (Humana Press, 2018).
10. National Institutes of Health. LiverTox: clinical and research information on drug-induced liver injury. *Nih.gov* https://livertox.nih.gov (2017).
11. Steele, M. A., Burk, R. F. & DesPrez, R. M. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* **99**, 465–471 (1991).
12. Perdices, E. V. et al. Hepatotoxicity associated with statin use: analysis of the cases included in the Spanish Hepatotoxicity Registry. *Rev. Esp. enfermedades Dig.* **106**, 246–254 (2014).
13. Björnsson, E. S., Bergmann, O. M., Björnsson, H. K., Kvaran, R. B. & Olafsson, S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. *Gastroenterology* **144**, 1419–1425 (2013).
A population-based study of the incidence and causes of DILI, and the first study to show the risk of DILI with the use of individual agents.
14. Björnsson, E., Jacobsen, E. I. & Kalaitzakis, E. Hepatotoxicity associated with statins: reports of idiosyncratic liver injury post-marketing. *J. Hepatol.* **56**, 374–380 (2012).
15. Chalasani, N. et al. Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. *Gastroenterology* **148**, 1340–1352 (2015).
16. Andrade, R. J. et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* **129**, 512–521 (2005).
The primary large publication from the Spanish Hepatotoxicity Registry, with the first large prospective study of patients with DILI.
17. Navarro, V. J. et al. Liver injury from herbals and dietary supplements in the U.S. Drug-Induced Liver Injury Network. *Hepatology* **60**, 1399–1408 (2014).
18. Andrade, R., Medina-Caliz, I., Gonzalez-Jimenez, A., Garcia-Cortes, M. & Lucena, M. I. Hepatic damage by natural remedies. *Semin. Liver Dis.* **38**, 021–040 (2018).
19. Suk, K. T. et al. A prospective nationwide study of drug-induced liver injury in Korea. *Am. J. Gastroenterol.* **107**, 1380–1387 (2012).
20. Shen, T. et al. Incidence and etiology of drug-induced liver injury in mainland China. *Gastroenterology* **156**, 2230–2241.e11 (2019).
21. World Health Organization. Traditional medicine strategy. 2014-2023. 76 Disponible en: https://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/ (Accedido: 30.ª enero 2019). (2013).
22. Aiso, M. et al. Analysis of 307 cases with drug-induced liver injury between 2010 and 2018 in Japan. *Hepatol. Res.* **49**, 105–110 (2019).
23. Zhou, Y. et al. Epidemiology of drug-induced liver injury in China: a systematic analysis of the Chinese literature including 21,789 patients. *Eur. J. Gastroenterol. Hepatol.* **25**, 825–829 (2013).
24. Wai, C.-T. et al. Drug-induced liver injury at an Asian center: a prospective study. *Liver Int.* **27**, 465–474 (2007).
25. Devarbhavi, H. Ayurvedic and herbal medicine-induced liver injury: it is time to wake up and take notice. *Indian J. Gastroenterol.* **37**, 5–7 (2018).
26. Wang, G.-Q., Deng, Y.-Q. & Hou, F.-Q. Overview of drug-induced liver injury in China. *Clin. Liver Dis.* **4**, 26–29 (2014).
27. Devarbhavi, H. et al. Single-center experience with drug-induced liver injury from India: causes, outcome, prognosis and predictors of mortality. *Am. J. Gastroenterol.* **105**, 2396–2404 (2010).
28. World Health Organization. Global tuberculosis report 2017. *Who.int* https://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf (2017).
29. Devarbhavi, H. et al. Drug-induced acute liver failure in children and adults: results of a single-centre study of 128 patients. *Liver Int.* **38**, 1322–1329 (2017).
30. de Abajo, F. J., Montero, D., Madurga, M. & Rodriguez, L. A. G. Acute and clinically relevant drug-induced liver injury: a population based case-control study. *Br. J. Clin. Pharmacol.* **58**, 71–80 (2004).
31. De Valle, M. B., Av Klinteberg, V., Alem, N., Olsson, R. & Björnsson, E. Drug-induced liver injury in a Swedish university hospital out-patient hepatology clinic. *Aliment. Pharmacol. Ther.* **24**, 1187–1195 (2006).
32. Sgro, C. et al. Incidence of drug-induced hepatic injuries: a French population-based study. *Hepatology* **36**, 451–455 (2002).
33. Björnsson, E. & Olsson, R. Outcome and prognostic markers in severe drug-induced liver disease. *Hepatology* **42**, 481–489 (2005).
34. Vega, M. et al. The incidence of drug- and herbal and dietary supplement-induced liver injury: preliminary findings from gastroenterologist-based surveillance in the population of the state of Delaware. *Drug Saf.* **40**, 783–787 (2017).
The first population-based study in the USA.
35. Goldberg, D. S. et al. Population-representative incidence of drug-induced acute liver failure based on an analysis of an integrated health care system. *Gastroenterology* **148**, 1353–61.e3 (2015).
36. Bessone, F. et al. When the creation of a consortium provides useful answers: experience of the Latin American DILI Network (LATINDILIN). *Clin. Liver Dis.* **13**, 51–57 (2019).
37. Amadi, C. & Orisakwe, O. Herb-induced liver injuries in developing nations: an update. *Toxics* **6**, 24 (2018).
38. Yuan, L. & Kaplowitz, N. Mechanisms of drug-induced liver injury. *Clin. Liver Dis.* **17**, 507–518 (2013).
39. Hagenbuch, B. & Stieger, B. The SLCO (former SLC21) superfamily of transporters. *Mol. Aspects Med.* **34**, 396–412 (2013).
40. Burckhardt, G. & Burckhardt, B. C. in *Drug Transporters. Handbook of Experimental Pharmacology, vol 201* (eds. Fromm, M. & Kim, R.) 29–104 (Springer, 2011).
41. Kullak-Ublick, G. A. et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**, 525–533 (2001).
42. Kovacsics, D., Patik, I. & Özvegy-Laczka, C. The role of organic anion transporting polypeptides in drug absorption, distribution, excretion and drug-drug interactions. *Expert Opin. Drug Metab. Toxicol.* **13**, 409–424 (2017).
43. Shitara, Y., Hirano, M., Sato, H. & Sugiyama, Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1: SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J. Pharmacol. Exp. Ther.* **311**, 228–236 (2004).
44. Khurana, V., Minocha, M., Pal, D. & Mitra, A. K. Inhibition of OATP-1B1 and OATP-1B3 by tyrosine kinase inhibitors. *Drug Metabol. Drug Interact.* **29**, 249–259 (2014).
45. Ellawatty, W. E. A. et al. Organic cation transporter 1 is responsible for hepatocellular uptake of the tyrosine kinase inhibitor pazopanib. *Drug Metab. Dispos.* **46**, 33–40 (2018).
46. Food and Drug Administration. Guidance document. In vitro metabolism- and transporter-mediated drug-drug interaction studies guidance for industry. *Fda.gov* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-metabolism-and-transporter-mediated-drug-drug-interaction-studies-guidance-industry (2017).
47. Park, B. K. et al. Managing the challenge of chemically reactive metabolites in drug development. *Nat. Rev. Drug Discov.* **10**, 292–306 (2011).
48. Weaver, R. J. et al. Test systems in drug discovery for hazard identification and risk assessment of human drug-induced liver injury. *Expert Opin. Drug Metab. Toxicol.* **13**, 767–782 (2017).

49. Daly, A. K. et al. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. *Gastroenterology* **132**, 272–281 (2007). Candidate gene study highlighting the role of SNPs in genes involved in drug metabolism and excretion.
50. Li, Y. et al. In vitro metabolic activation of lumiracoxib in rat and human liver preparations. *Drug Metab. Dispos.* **36**, 469–473 (2008).
51. He, K. et al. Metabolic activation of troglitazone: identification of a reactive metabolite and mechanisms involved. *Drug Metab. Dispos.* **32**, 639–646 (2004).
52. Kullak-Ublick, G. A. et al. Drug-induced liver injury: recent advances in diagnosis and risk assessment. *Gut* **66**, 1154–1164 (2017).
A state-of-the-art review highlighting recent advances in DILI diagnosis.
53. Stieger, B., Fattinger, K., Madon, J., Kullak-Ublick, G. A. & Meier, P. J. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (BSEP) of rat liver. *Gastroenterology* **118**, 422–430 (2000).
54. Krähenbühl, S., Talos, C., Fischer, S. & Reichen, J. Toxicity of bile acids on the electron transport chain of isolated rat liver mitochondria. *Hepatology* **19**, 471–479 (1994).
55. Tujios, S. & Fontana, R. J. Mechanisms of drug-induced liver injury: from bedside to bench. *Nat. Rev. Gastroenterol. Hepatol.* **8**, 202–211 (2011).
56. Fattinger, K. et al. The endothelin antagonist bosentan inhibits the canalicular bile salt export pump: a potential mechanism for hepatic adverse reactions. *Clin. Pharmacol. Ther.* **69**, 223–231 (2001).
57. Morgan, R. E. et al. Interference with bile salt export pump function is a susceptibility factor for human liver injury in drug development. *Toxicol. Sci.* **118**, 485–500 (2010).
58. Dawson, S., Stahl, S., Paul, N., Barber, J. & Kenna, J. G. In vitro inhibition of the bile salt export pump correlates with risk of cholestatic drug-induced liver injury in humans. *Drug Metab. Dispos.* **40**, 130–138 (2012).
59. Funk, C., Ponelle, C., Scheuermann, G. & Pantze, M. Cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity: in vivo and in vitro interaction at the canalicular bile salt export pump (Bsep) in the rat. *Mol. Pharmacol.* **59**, 627–635 (2001).
60. Aleo, M. D. et al. Human drug-induced liver injury severity is highly associated with dual inhibition of liver mitochondrial function and bile salt export pump. *Hepatology* **60**, 1015–1022 (2014).
61. Keppler, D. Multidrug resistance proteins (MRPs, ABCCs): importance for pathophysiology and drug therapy. *Handb. Exp. Pharmacol.* **201**, 299–323 (2011).
62. Choi, J. H. et al. MRP2 haplotypes confer differential susceptibility to toxic liver injury. *Pharmacogenet. Genomics* **17**, 403–415 (2007).
63. Ulzurrun, E. et al. Role of chemical structures and the 1331T>C bile salt export pump polymorphism in idiosyncratic drug-induced liver injury. *Liver Int.* **33**, 1378–1385 (2013).
64. Miura, Y. et al. Sunitinib-induced severe toxicities in a Japanese patient with the ABCG2 421 AA genotype. *BMC Cancer* **14**, 964 (2014).
65. Lang, C. et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. *Pharmacogenet. Genomics* **17**, 47–60 (2007).
66. Yoshikado, T. et al. Itraconazole-induced cholestasis: involvement of the inhibition of bile canalicular phospholipid translocator MDR3/ABCB4. *Mol. Pharmacol.* **79**, 241–250 (2011).
67. Win, S., Than, T. A., Min, R. W. M., Aghajan, M. & Kaplowitz, N. c-Jun N-terminal kinase mediates mouse liver injury through a novel Sab (SH3BP5)-dependent pathway leading to inactivation of intramitochondrial Src. *Hepatology* **63**, 1987–2003 (2016).
68. Iorga, A., Dara, L. & Kaplowitz, N. Drug-induced liver injury: cascade of events leading to cell death, apoptosis or necrosis. *Int. J. Mol. Sci.* **18**, 1018 (2017).
69. Roth, R. A., Maiuri, A. R. & Ganey, P. E. Idiosyncratic drug-induced liver injury: is drug-cytokine interaction the linchpin? *J. Pharmacol. Exp. Ther.* **360**, 461–470 (2017).
70. Jaeschke, H., Williams, C. D., Ramachandran, A. & Bajt, M. L. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liver Int.* **32**, 8–20 (2012).
71. Jaeschke, H. Innate immunity and acetaminophen-induced liver injury: why so many controversies? *Hepatology* **48**, 699–701 (2008).
72. Dara, L. et al. Receptor interacting protein kinase 1 mediates murine acetaminophen toxicity independent of the necrosome and not through necroptosis. *Hepatology* **62**, 1847–1857 (2015).
73. Luedde, T., Kaplowitz, N. & Schwabe, R. F. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. *Gastroenterology* **147**, 765–783.e4 (2014).
74. Yip, L. Y. et al. The liver-gut microbiota axis modulates hepatotoxicity of tacrine in the rat. *Hepatology* **67**, 282–295 (2018).
75. Gong, S. et al. Gut microbiota mediates diurnal variation of acetaminophen induced acute liver injury in mice. *J. Hepatol.* **69**, 51–59 (2018).
76. Monshi, M. M. et al. Human leukocyte antigen (HLA)-B*57:01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology* **57**, 727–739 (2013).
77. Grove, J. I. & Aithal, G. P. Human leukocyte antigen genetic risk factors of drug-induced liver toxicology. *Expert Opin. Drug Metab. Toxicol.* **11**, 395–409 (2015).
78. Kaplowitz, N. Avoiding idiosyncratic DILI: two is better than one. *Hepatology* **58**, 15–17 (2013).
79. Light, D. S., Aleo, M. D. & Kenna, J. G. in *Drug-Induced Liver Toxicity. Methods in Pharmacology and Toxicology* (eds Chen, M. & Will, Y.) 345–364 (Humana Press, 2018).
80. Kenna, J. G. et al. Can bile salt export pump inhibition testing in drug discovery and development reduce liver injury risk? an international transporter consortium perspective. *Clin. Pharmacol. Ther.* **104**, 916–932 (2018).
81. Uetrecht, J. & Kaplowitz, N. Inhibition of immune tolerance unmasks drug-induced allergic hepatitis. *Hepatology* **62**, 346–348 (2015).
82. Russmann, S., Jetter, A. & Kullak-Ublick, G. A. Pharmacogenetics of drug-induced liver injury. *Hepatology* **52**, 748–761 (2010).
83. Institute of Medicine. *Exploring the Biological Contributions to Human Health: Does Sex Matter?* (National Academies Press, 2001).
84. Mennecozzi, M., Landesmann, B., Palosaari, T., Harris, G. & Whelan, M. Sex differences in liver toxicity—do female and male human primary hepatocytes react differently to toxicants in vitro? *PLoS One* **10**, e0122786 (2015).
85. Xie, Y. et al. Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. *Toxicol. Appl. Pharmacol.* **279**, 266–274 (2014).
86. Dai, G., He, L., Chou, N. & Wan, Y.-J. Y. Acetaminophen metabolism does not contribute to gender difference in its hepatotoxicity in mouse. *Toxicol. Sci.* **92**, 33–41 (2006).
87. Sheng, Y., Liang, Q., Deng, Z., Ji, L. & Wang, Z. Acetaminophen induced gender-dependent liver injury and the involvement of GCL and GPx. *Drug Discov. Ther.* **7**, 78–83 (2013).
88. Du, K., Williams, C. D., McGill, M. R. & Jaeschke, H. Lower susceptibility of female mice to acetaminophen hepatotoxicity: role of mitochondrial glutathione, oxidant stress and c-jun N-terminal kinase. *Toxicol. Appl. Pharmacol.* **281**, 58–66 (2014).
89. Leibman, D., Smolen, A. & Smolen, T. N. Strain, sex and developmental profiles of cocaine metabolizing enzymes in mice. *Pharmacol. Biochem. Behav.* **37**, 161–165 (1990).
90. Visalli, T., Turkall, R. & Abdel-Rahman, M. S. Gender differences in cocaine pharmacokinetics in CF-1 mice. *Toxicol. Lett.* **155**, 35–40 (2005).
91. You, Q., Cheng, L., Reilly, T. P., Wegmann, D. & Ju, C. Role of neutrophils in a mouse model of halothane-induced liver injury. *Hepatology* **44**, 1421–1431 (2006).
92. Dugan, C. M., Fullerton, A. M., Roth, R. A. & Ganey, P. E. Natural killer cells mediate severe liver injury in a murine model of halothane hepatitis. *Toxicol. Sci.* **120**, 507–518 (2011).
93. Toyoda, Y. et al. Mechanism of exacerbative effect of progesterone on drug-induced liver injury. *Toxicol. Sci.* **126**, 16–27 (2012).
94. Toyoda, Y. et al. Estradiol and progesterone modulate halothane-induced liver injury in mice. *Toxicol. Lett.* **204**, 17–24 (2011).
95. Cho, J. et al. Sex bias in experimental immune-mediated, drug-induced liver injury in BALB/c mice: suggested roles for Tregs, estrogen, and IL-6. *PLoS One* **8**, e61186 (2013).
96. George, N., Chen, M., Yuen, N., Hunt, C. M. & Suzuki, A. Interplay of gender, age and drug properties on reporting frequency of drug-induced liver injury. *Regul. Toxicol. Pharmacol.* **94**, 101–107 (2018).
97. Hunt, C. M., Yuen, N. A., Stirnadel-Farrant, H. A. & Suzuki, A. Age-related differences in reporting of drug-associated liver injury: data-mining of WHO Safety Report Database. *Regul. Toxicol. Pharmacol.* **70**, 519–526 (2014).
98. Lucena, M. I. et al. Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. *Hepatology* **49**, 2001–2009 (2009).
99. Suzuki, A. et al. Associations of gender and a proxy of female menopausal status with histological features of drug-induced liver injury. *Liver Int.* **37**, 1723–1730 (2017).
100. Gonzalez-Jimenez, A. et al. The influence of drug properties and host factors on delayed onset of symptoms in drug-induced liver injury. *Liver Int.* **39**, 401–410 (2019).
101. Ortega-Alonso, A., Stephens, C., Lucena, M. I. & Andrade, R. J. Case characterization, clinical features and risk factors in drug-induced liver injury. *Int. J. Mol. Sci.* **17**, 714 (2016).
102. Kaliyaperumal, K. et al. Pharmacogenomics of drug-induced liver injury (DILI): molecular biology to clinical applications. *J. Hepatol.* **69**, 948–957 (2018).
An up-to-date review of genetic susceptibility to DILI in a clinical context.
103. Devarbhavi, H. & Raj, S. Drug-induced liver injury with skin reactions: drugs and host risk factors, clinical phenotypes and prognosis. *Liver Int.* **39**, 802–811 (2019).
104. Devarbhavi, H., Karanth, D., Prasanna, K. S., Adarsh, C. K. & Patil, M. Drug-induced liver injury with hypersensitivity features has a better outcome: a single-center experience of 39 children and adolescents. *Hepatology* **54**, 1344–1350 (2011).
105. Devarbhavi, H., Raj, S., Joseph, T., Singh, R. & Patil, M. Features and treatment of dapsone-induced hepatitis, based on analysis of 44 cases and literature review. *Clin. Gastroenterol. Hepatol.* **15**, 1805–1807 (2017).
106. Kardaun, S. H. et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br. J. Dermatol.* **156**, 609–611 (2007).
107. Bastuji-Garin, S. et al. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch. Dermatol.* **129**, 92–96 (1993).
108. Czaja, A. J. Drug-induced autoimmune-like hepatitis. *Dig. Dis. Sci.* **56**, 958–976 (2011).
109. Devarbhavi, H. et al. Drug-induced liver injury associated with Stevens-Johnson syndrome/toxic epidermal necrolysis: patient characteristics, causes, and outcome in 36 cases. *Hepatology* **63**, 993–999 (2016).
110. Aithal, G. P. et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin. Pharmacol. Ther.* **89**, 806–815 (2011).
This paper established international consensus on case definitions and phenotypic charecterization of DILI.
111. Senior, J. R. Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury–past, present, and future. *Clin. Pharmacol. Ther.* **92**, 332–339 (2012).
112. Dara, L., Liu, Z.-X. & Kaplowitz, N. Mechanisms of adaptation and progression in idiosyncratic drug induced liver injury, clinical implications. *Liver Int.* **36**, 158–165 (2016).
A description of various factors that contribute to pathogenesis of idiosyncratic DILI, highlighting the participation of genetic susceptibility due to HLA variants and the potential importance of immune tolerance.
113. Chalasani, N. et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology* **135**, 1924–1934.e4 (2008).
114. Davern, T. J. et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology* **141**, 1665–1672 (2011).
115. Sanjuan-Jimenez, R. et al. Prevalence of hepatitis E markers in Spanish patients with suspected drug-induced liver injury [abstract]. *Hepatology* **66**, 423A (2017).
116. Dalton, H. R. et al. EASL Clinical Practice Guidelines on hepatitis E virus infection. *J. Hepatol.* **68**, 1256–1271 (2018).
117. Suzuki, A. et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. *Hepatology* **54**, 931–939 (2011).
118. Foureau, D. M. et al. Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced

liver injury, idiopathic autoimmune and viral hepatitis. *Clin. Exp. Immunol.* **180**, 40–51 (2015).
119. Kleiner, D. E. et al. Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. *Hepatology* **59**, 661–670 (2014).
120. Bonkovsky, H. L. et al. Clinical presentations and outcomes of bile duct loss caused by drugs and herbal and dietary supplements. *Hepatology* **65**, 1267–1277 (2017).
121. Andrade, R. J., Robles, M. & Lucena, M. I. Rechallenge in drug-induced liver injury: the attractive hazard. *Expert Opin. Drug Saf.* **8**, 709–714 (2009).
122. García-Cortés, M., Stephens, C., Lucena, M. I., Fernández-Castañer, A. & Andrade, R. J. Causality assessment methods in drug induced liver injury: strengths and weaknesses. *J. Hepatol.* **55**, 683–691 (2011).
A comprehensive review on the shortcomings of the liver-specific CIOMS/RUCAM scale.
123. García-Cortés, M. et al. Evaluation of Naranjo Adverse Drug Reactions Probability Scale in causality assessment of drug-induced liver injury. *Aliment. Pharmacol. Ther.* **27**, 780–789 (2008).
124. Danan, G. & Benichou, C. Causality assessment of adverse reactions to drugs—I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J. Clin. Epidemiol.* **46**, 1323–1330 (1993).
125. Lucena, M., Camargo, R., Andrade, R. J., Perez-Sanchez, C. J. & Sanchez De La Cuesta, F. Comparison of two clinical scales for causality assessment in hepatotoxicity. *Hepatology* **33**, 123–130 (2001).
126. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: drug-induced liver injury. *J Hepatol.* **70**, 1222-1261 (2019).
A comprehensive review of DILI and recommendations for clinical practice.
127. Rochon, J. et al. Reliability of the Roussel Uclaf Causality Assessment Method for assessing causality in drug-induced liver injury. *Hepatology* **48**, 1175–1183 (2008).
128. Navarro, V. J. et al. Liver injury from herbal and dietary supplements. *Hepatology* **65**, 363–373 (2017).
129. Suzman, D. L., Pelosof, L., Rosenberg, A. & Avigan, M. I. Hepatotoxicity of immune checkpoint inhibitors: an evolving picture of risk associated with a vital class of immunotherapy agents. *Liver Int.* **38**, 976–987 (2018).
130. Rockey, D. C. et al. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality assessment method. *Hepatology* **51**, 2117–2126 (2010).
131. Hayashi, P. H. Drug-induced liver injury network causality assessment: criteria and experience in the United States. *Int. J. Mol. Sci.* **17**, 201 (2016).
132. Hayashi, P. H. et al. Reliability of causality assessment for drug, herbal and dietary supplement hepatotoxicity in the Drug-Induced Liver Injury Network (DILIN). *Liver Int.* **35**, 1623–1632 (2015).
133. Takikawa, H. et al. Assessment of 287 Japanese cases of drug induced liver injury by the diagnostic scale of the International Consensus Meeting. *Hepatol. Res.* **27**, 192–195 (2003).
134. Whritenour, J. et al. Development of a modified lymphocyte transformation test for diagnosing drug-induced liver injury associated with an adaptive immune response. *J. Immunotoxicol.* **14**, 31–38 (2017).
135. Benesic, A. et al. Development and validation of a test to identify drugs that cause idiosyncratic drug-induced liver injury. *Clin. Gastroenterol. Hepatol.* **16**, 1488–1494.e5 (2018).
136. Danan, G. & Teschke, R. RUCAM in drug and herb induced liver injury: the update. *Int. J. Mol. Sci.* **17**, 14 (2015).
137. Church, R. J. & Watkins, P. B. The transformation in biomarker detection and management of drug-induced liver injury. *Liver Int.* **37**, 1582–1590 (2017).
138. Food and Drug Administration. FDA letter of support for drug-induced liver injury (DILI) biomarkers. *Fda.gov* https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/UCM517355.pdf (2016).
139. Hu, J. et al. MiR-122 in hepatic function and liver diseases. *Protein Cell* **3**, 364–371 (2012).
140. Church, R. J. et al. Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: an international collaborative effort. *Hepatology* **69**, 760–763 (2019).
141. Rivkin, M. et al. Inflammation-induced expression and secretion of microRNA 122 leads to reduced blood levels of kidney-derived erythropoietin and anemia. *Gastroenterology* **151**, 999–1010.e3 (2016).
142. Chai, C. et al. Metabolic circuit involving free fatty acids, microRNA 122, and triglyceride synthesis in liver and muscle tissues. *Gastroenterology* **153**, 1404–1415 (2017).
143. Church, R. J. & Watkins, P. B. Reply. *Hepatology* **67**, 2481–2482 (2018).
144. Schmidt, E. S. & Schmidt, F. W. Glutamate dehydrogenase: biochemical and clinical aspects of an interesting enzyme. *Clin. Chim. Acta.* **173**, 43–55 (1988).
145. Russo, M. W. et al. Profiles of miRNAs in serum in severe acute drug induced liver injury and their prognostic significance. *Liver Int.* **37**, 757–764 (2017).
146. Steuerwald, N. M. et al. Profiles of serum cytokines in acute drug-induced liver injury and their prognostic significance. *PLoS One* **8**, e81974 (2013).
147. Zimmerman, H. J. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. (Lippincott Williams and Wilkins, 1999).
148. Temple, R. Hy's law: predicting serious hepatotoxicity. *Pharmacoepidemiol. Drug Saf.* **15**, 241–243 (2006).
149. Senior, J. R. Evolution of the Food and Drug Administration approach to liver safety assessment for new drugs: current status and challenges. *Drug Saf.* **37** (Suppl 1), S9–S17 (2014).
An important paper from the regulatory perspective of the risk of DILI with new compounds.
150. Robles–Diaz, M. et al. Use of Hy's law and a new composite algorithm to predict acute liver failure in patients with drug-induced liver injury. *Gastroenterology* **147**, 109–118 (2014).
151. Björnsson, E., Nordlinder, H. & Olsson, R. Clinical characteristics and prognostic markers in disulfiram-induced liver injury. *J. Hepatol.* **44**, 791–797 (2006).
152. Pachkoria, K. et al. Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. *J. Hepatol.* **49**, 107–114 (2008).
153. Medina-Caliz, I. et al. Definition and risk factors for chronicity following acute idiosyncratic drug-induced liver injury. *J. Hepatol.* **65**, 532–542 (2016).
154. Hayashi, P. H. et al. Death and liver transplantation within 2 years of onset of drug-induced liver injury. *Hepatology* **66**, 1275–1285 (2017).
155. García-Cortés, M., Ortega-Alonso, A., Lucena, M. I. & Andrade, R. J. Drug-induced liver injury: a safety review. *Expert Opin. Drug Saf.* **17**, 795–804 (2018).
156. Regev, A. Drug-induced liver injury and drug development: industry perspective. *Semin. Liver Dis.* **34**, 227–239 (2014).
157. Food and Drug Administration. Guidance document. Drug-induced liver injury: premarketing clinical evaluation. *Fda.gov* https://www.fda.gov/downloads/Guidances/UCM174090.pdf (2009).
A key guidance document for industry on detecting, assessing and managing liver toxicity in clinical trials.
158. Newsome, P. N. et al. Guidelines on the management of abnormal liver blood tests. *Gut* **67**, 6–19 (2018).
159. Ozer, J., Ratner, M., Shaw, M., Bailey, W. & Schomaker, S. The current state of serum biomarkers of hepatotoxicity. *Toxicology* **245**, 194–205 (2008).
160. Hunt, C. M., Forster, J. K., Papay, J. I. & Stirnadel, H. A. Evidence-based liver chemistry monitoring in drug development. *Pharmaceut. Med.* **23**, 149–158 (2009).
A retrospective analysis of time-to-onset, incidence, phenotype and severity of liver injury in clinical trials with recommendation on monitoring intervals by development stage.
161. Lee, W. M. Drug-induced hepatotoxicity. *N. Engl. J. Med.* **349**, 474–485 (2003).
162. Devarbhavi, H. An update on drug-induced liver Injury. *J. Clin. Exp. Hepatol.* **2**, 247–259 (2012).
163. Kaplowitz, N. Rules and laws of drug hepatotoxicity. *Pharmacoepidemiol. Drug Saf.* **15**, 231–233 (2006).
164. Guo, T., Senior, J., Gelperin, K. & Food and Drug Administration. How a SAS/IntrNet tool was created at the FDA for the detection of potential drug-induced liver injury using data with CDISC standard. *Lexjansen.com* https://www.lexjansen.com/wuss/2009/cdi/CDI-Guo.pdf (2008).
165. Watkins, P. B. et al. Evaluation of drug-induced serious hepatotoxicity (eDISH): application of this data organization approach to phase III clinical trials of rivaroxaban after total hip or knee replacement surgery. *Drug Saf.* **34**, 243–252 (2011).
166. Merz, M., Lee, K. R., Kullak-Ublick, G. A., Brueckner, A. & Watkins, P. B. Methodology to assess clinical liver safety data. *Drug Saf.* **37**, 33–45 (2014).
167. Graham, D. J., Green, L., Senior, J. R. & Nourjah, P. Troglitazone-induced liver failure: a case study. *Am. J. Med.* **114**, 299–306 (2003).
168. Graham, D. J., Drinkard, C. R. & Shatin, D. Incidence of idiopathic acute liver failure and hospitalized liver injury in patients treated with troglitazone. *Am. J. Gastroenterol.* **98**, 175–179 (2003).
169. Graham, D. J., Drinkard, C. R., Shatin, D., Tsong, Y. & Burgess, M. J. Liver enzyme monitoring in patients treated with troglitazone. *JAMA* **286**, 831–833 (2001).
170. Chalasani, N. P. et al. ACG clinical guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. *Am. J. Gastroenterol.* **109**, 950–966 (2014).
The first guidelines for the diagnosis and management of DILI.
171. Puzanov, I. et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J. Immunother. Cancer* **5**, 95 (2017).
172. Abu-Sbeih, H., Styskel, B., Blechazz, B., Chalasani, N. & Miller, E. Clinically significant hepatotoxicity due to immune checkpoint inhibitors is rare but leads to treatment discontinuations in high proportion. *Hepatology* **68**, 25A (2018).
173. De Martin, E. et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. *J. Hepatol.* **68**, 1181–1190 (2018).
174. Gauci, M.-L. et al. Immune-related hepatitis with immunotherapy: are corticosteroids always needed? *J. Hepatol.* **69**, 548–550 (2018).
175. Lee, W. M. et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. *Gastroenterology* **137**, 856–864 (2009).
176. Butt, T. F., Cox, A. R., Oyebode, J. R. & Ferner, R. E. Internet accounts of serious adverse drug reactions: a study of experiences of Stevens-Johnson syndrome and toxic epidermal necrolysis. *Drug Saf.* **35**, 1159–1170 (2012).
177. Kowski, A. B. et al. Specific adverse effects of antiepileptic drugs–a true-to-life monotherapy study. *Epilepsy Behav.* **54**, 150–157 (2016).
178. Ware, J. E. & Sherbourne, C. D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med. Care* **30**, 473–483 (1992).
179. Horne, R., Weinman, J. & Hankins, M. The beliefs about medicines questionnaire: the development and evaluation of a new method for assessing the cognitive representation of medication. *Psychol. Heal.* **14**, 1–24 (1999).
180. Suh, J. I. et al. Anxiety and depression propensities in patients with acute toxic liver injury. *World J. Gastroenterol.* **19**, 9069–9076 (2013).
181. Fontana, R. J. et al. Persistent liver biochemistry abnormalities are more common in older patients and those with cholestatic drug induced liver injury. *Am. J. Gastroenterol.* **110**, 1450–1459 (2015).
182. Ostapowicz, G. et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann. Intern. Med.* **137**, 947–954 (2002).
183. Rangnekar, A. S. et al. Quality of life is significantly impaired in long-term survivors of acute liver failure and particularly in acetaminophen-overdose patients. *Liver Transpl.* **19**, 991–1000 (2013).
184. Rzouq, F. S. et al. Hepatotoxicity fears contribute to underutilization of statin medications by primary care physicians. *Am. J. Med. Sci.* **340**, 89–93 (2010).
185. Watkins, P. B. The DILI-sim initiative: insights into hepatotoxicity. mechanisms and biomarker interpretation. *Clin. Transl. Sci.* **12**, 122–129 (2019).
This is a review of a 15 year public–private partnership that has been using quantitative systems toxicology approaches to understand and predict the likelihood that a new drug candidate will cause DILI. The review focuses on specific advances in mechanistic understanding that have evolved from this consortium.
186. Aithal, G. P. Pharmacogenetic testing in idiosyncratic drug-induced liver injury: current role in clinical practice. *Liver Int.* **35**, 1801–1808 (2015).
187. Alvarez, F. et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* **31**, 929–938 (1999).

188. Kowalec, K. et al. Common variation near IRF6 is associated with IFN-β-induced liver injury in multiple sclerosis. *Nat. Genet.* **50**, 1081–1085 (2018).
189. Cirulli, E. T. et al. A missense variant in PTPN22 is a risk factor for drug-induced liver injury. *Gastroenterology* **156**, 1707–1716.e2 (2019).
190. Kozyra, M., Ingelman-Sundberg, M. & Lauschke, V. M. Rare genetic variants in cellular transporters, metabolic enzymes, and nuclear receptors can be important determinants of interindividual differences in drug response. *Genet. Med.* **19**, 20–29 (2017).
191. Khera, A. V. et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat. Genet.* **50**, 1219–1224 (2018).
192. Metushi, I. G., Hayes, M. A. & Uetrecht, J. Treatment of PD-1(-/-) mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. *Hepatology* **61**, 1332–1342 (2015).
193. Fontana, R. J. et al. Idiosyncratic drug-induced liver injury is associated with substantial morbidity and mortality within 6 months from onset. *Gastroenterology* **147**, 96–108 (2014).
194. Jaeschke, H. & McGill, M. R. Serum glutamate dehydrogenase—biomarker for liver cell death or mitochondrial dysfunction? *Toxicol. Sci.* **134**, 221–222 (2013).
195. Francis, B. et al. Temporary removal: reference intervals for putative biomarkers of drug-induced liver injury and liver regeneration in healthy human volunteers. *J. Hepatol.* https://doi.org/10.1016/j.jhep.2018.04.024 (2018).
196. Suzuki, A. et al. Comedications alter drug-induced liver injury reporting frequency: data mining in the WHO VigiBase™. *Regul. Toxicol. Pharmacol.* **72**, 481–490 (2015).
197. Suzuki, A. et al. Co-medications that modulate liver injury and repair influence clinical outcome of acetaminophen-associated liver injury. *Clin. Gastroenterol. Hepatol.* **7**, 882–888 (2009).
198. Bianchi, I., Lleo, A., Gershwin, M. E. & Invernizzi, P. The X chromosome and immune associated genes. *J. Autoimmun.* **38**, J187–J192 (2012).
199. Gianesin, K. et al. Premature aging and immune senescence in HIV-infected children. *AIDS* **30**, 1363–1373 (2016).
200. Nakajima, T. et al. Premature telomere shortening and impaired regenerative response in hepatocytes of individuals with NAFLD. *Liver Int.* **26**, 23–31 (2006).

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