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Drug-Induced Liver Injury

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Abstract

Drug-induced liver injury is a frequent cause of hepatic dysfunction. Reliably establishing whether the liver disease was caused by a drug requires the exclusion of other plausible causes and the search for a clinical drug signature. The drug signature consists of the pattern of liver test abnormality, the duration of latency to symptomatic presentation, the presence or absence of immune-mediated hypersensitivity and the response to drug withdrawal.

Determination of causality also includes an evaluation of individual susceptibility to drug-induced liver injury. This susceptibility is governed by both genetic and environmental factors. Components of the drug signature in conjunction with certain risk factors have been incorporated into formal scoring systems that are predictive of the likelihood of drug-induced liver injury. The most validated

scoring system is the Roussel-Uclaf causality assessment method, which nonetheless retains certain imperfections.

Mitigating the potential for drug-induced liver injury is achieved by the identification of toxicity signals during clinical trials and the monitoring of liver tests in clinical practice. There are three signals of liver toxicity in clinical trials: (i) a statistically significant doubling (or more) in the incidence of serum alanine aminotransferase (ALT) elevation >3 × the upper limit of normal (ULN); (ii) any incidence of serum ALT elevation >8–10 × ULN; and (iii) any incidence of serum ALT elevation >3 × ULN accompanied by a serum bilirubin elevation >2 × ULN. Monitoring of liver tests in clinical practice has shown unconvincing efficacy, but where a benefit-risk analysis would favour continued therapy, monthly monitoring may have some benefit compared with no monitoring at all.

With rare exception, treatment of drug-induced liver injury is principally supportive. Drug toxicity is the most common cause of acute liver failure, defined as a prolonged prothrombin time (international normalised ratio ≥1.5) and any degree of mental alteration occurring <26 weeks after the onset of illness in a patient without pre-existing cirrhosis. A patient who meets these criteria must be evaluated for liver transplantation. The pathogenesis of drug-induced liver injury can be examined on the basis of the two principal patterns of injury. The hepatocellular pattern is characterised by a predominant rise in the level of transaminases and results from the demise of hepatocytes by means of either apoptosis or necrosis. The cholestatic pattern is characterised by a predominant rise of the serum alkaline phosphatase level and usually results from injury to the bile ductular cells either directly by the drug or its metabolite, or indirectly by an adaptive immune response.

Drug-induced liver injury represents a clinical challenge owing to the large number of reported hepatotoxic drugs in current use, the broad spectrum of hepatic injury by which it may become manifest and the frequent absence of clinical findings that permit its diagnosis with certitude.

Liver injury has been linked to nearly 1000 drugs[1] and is the single most frequent reason for removing approved medications from the market.[2] Furthermore, it accounts for more than half of the cases of acute liver failure, with paracetamol (acetaminophen) being the principal offending agent.[3] The annual percentage of paracetamol-related acute liver failure has risen from 28% in 1998 to 51% in 2003.[4] However, it remains unclear if this represents a true increase in paracetamol-related liver failure or a decrease in other causes of acute liver failure such as viral hepatitis. Among the non-paracetamol cases of acute liver failure due to drug-induced liver injury, antibacterials, particularly antituberculous drugs, are the major responsible class.[5]

Nonetheless, the incidence and seriousness of drug-induced liver injury remains largely underestimated in the general population.[6] A recent call to awareness was published, demonstrating that approximately 1 in 100 patients admitted to a medicine service develops drug-induced liver injury during the course of hospitalisation.[7] The present review provides a practical approach to the diagnosis and management of patients with suspected drug-related liver disease and includes a discussion of the most current concepts relating to pathogenesis.

1. Clinical Overview

Although it usually resembles either acute hepatitis, cholestatic liver disease or mixed hepatitis/cholestasis (table I), drug-induced liver injury can mimic all forms of acute and chronic hepatobiliary

Table I. Examples of hepatitis and cholestasis/mixed patterns of drug-induced liver injury

Pattern of liver injury[a]	Type	Examples
Hepatitis	Immune-mediated[b]	Allopurinol; diclofenac[c]; dihydralazine; germander; halothane; methyldopa; minocycline; nevirapine; nitrofurantoin; phenytoin; propylthiouracil; tienilic acid; trovafloxacin
	Non-immune-mediated[d]	Acarbose; amiodarone; bosentan; dantrolene; diclofenac[c]; disulfiram; felbamate; flutamide; HAART therapy; HMG-CoA reductase inhibitors ('statins'); isoniazid; ketoconazole; labetalol; leflunomide; methotrexate; nefazodone; nevirapine; nicotinic acid; paracetamol (acetaminophen); pemoline; pyrazinamide; rifampicin (rifampin); tacrine; tolcapone; troglitazone; valproate sodium; ximelagatran; zafirlukast; zileutin
Cholestasis/mixed	Immune-mediated	ACE inhibitors; amitriptyline; amoxicillin/clavulanic acid; carbamazepine; chlorpromazine; cotrimoxazole (trimethoprim/sulfamethoxazole); erythromycins; phenobarbital; sulfonamides; sulindac; tricyclic antidepressants
	Non-immune-mediated	Anabolic steroids; azathioprine; ciclosporin (cyclosporine); estrogens; oral contraceptives; terbinafine

a Please refer to section 2.2.1 for definitions of the three types of liver injury.

b Immune-mediated: may be characterised by fever, rash, eosinophilia or autoantibodies; rapid positive re-challenge occurs in variable proportion of cases.

c Reaction to diclofenac may be either immune-mediated or non-immune mediated.

d Non-immune mediated: not characterised by fever, rash, eosinophilia or autoantibodies.

HAART = highly active antiretroviral therapy.

diseases[8] (table II). Hence, the possibility of drug toxicity should be considered in all patients with hepatic dysfunction.

Establishing a definitive diagnosis of drug-induced liver injury remains, to date, an impossibility in most cases. Even histological analysis permits only the recognition of the type and degree of injury, rather than indicating that the injury is resultant from a specific drug. However, the potential exists for exploiting the response of the immune system to assist in the diagnosis of drug-induced liver injury. For instance, the presence of autoantibodies to specific cytochrome P450 (CYP) alleles has been associated with hypersensitivity reactions to certain drugs (table III).[9-12] Detection assays for these antibodies may prove to have diagnostic value, but their use is currently restricted to research laboratories. These autoantibodies may also be seen in drug-exposed patients without concomitant drug-induced liver injury. In addition, lymphocyte-stimulation tests may be used to identify the participation of the adaptive immune system in allergic reactions. The test involves exposure of peripheral blood mononuclear cells from the patient to the drug, and subsequent determination of lymphocyte proliferation using radiolabelled thymidine incorporation.[13,14] This approach has not gained favour in the US, perhaps because of the lack of standardisation and reproducibility. A new assay for the detection of

Table II. Other manifestations of drug-induced liver injury

Type of injury	Causative drugs
Fibrosis/cirrhosis	Methotrexate
Granulomas	Allopurinol; amoxicillin/clavulanic acid; carbamazepine; hydralazine; methyldopa; penicillamine; phenylbutazone; phenytoin; procainamide; quinidine; sulfonamides
Microvesicular steatosis	NRTIs; valproate sodium
Neoplasms	
adenoma	Anabolic steroids; oral contraceptives
angiosarcoma	Anabolic steroids
cholangiocarcinoma	Anabolic steroids
hepatocellular carcinoma	Danazol
Non-alcoholic steatohepatitis	Amiodarone; tamoxifen
Phospholipidosis	Amiodarone
Vascular lesions	
Budd-Chiari syndrome	Oral contraceptives
peliosis hepatis	Anabolic steroids; azathioprine; oral contraceptives
perisinusoidal fibrosis	Retinol (vitamin A)
veno-occlusive disease	Busulfan; cyclophosphamide

NRTIs = nucleoside reverse transcriptase inhibitors.

serum paracetamol adducts may prove useful in identifying cases lacking historical or other clinical data for both adults[15] and children.[16]

At present, the one recourse at our disposal is the capacity to ascertain the likelihood of drug-induced liver injury on the basis of circumstantial evidence. Gathering this evidence is a two-step process, requiring first the rigorous exclusion of other aetiologies of liver injury and second the identification of a drug-specific clinical signature. This clinical signature is constituted by the: (i) pattern of liver test abnormality; (ii) duration of latency to symptomatic presentation; (iii) presence or absence of immune-mediated hypersensitivity; and (iv) response to drug withdrawal. However, it should be noted that in certain cases, there may be different clinical presentations for the same drug.

2. Diagnosis

2.1 Exclusion of Other Aetiologies of Liver Injury

The diagnosis of drug-induced liver injury remains one of exclusion and, consequently, a work-up for other causes of hepatobiliary disease should be concurrently pursued. In the appropriate clinical setting, consideration should be given to viral hepatitis, to sepsis-induced cholestasis and to alcoholic, autoimmune, biliary, haemodynamic and metabolic disorders (table IV). The composition of total parenteral nutrition should also be investigated since the administration of carbohydrate-rich formulas is often complicated by steatohepatitis.[17,18] Non-hepatic causes of abnormal liver chemistries should also be sought (table V).

2.2 Clinical Signature of the Drug

2.2.1 Pattern of Liver Test Abnormality

On the basis of the alanine aminotransferase (ALT) and alkaline phosphatase (AP) levels, the liver test abnormalities are divisible into either acute hepatitis, cholestasis or a mixed hepatitis/cholestatis pattern. The classification scheme for these injury patterns was first established by the CIOMS,[19] and has been recently modified by the US FDA Drug Hepatotoxicity Steering Committee.[20] The current classification is established as follows (where ULN signifies upper limit of normal):

- Hepatitis (equation 1):

$$\text{ALT} \geq 3 \times \text{ULN} \textbf{ and } \frac{(\text{ALT/ULN})}{(\text{AP/ULN})} \geq 5 \quad \text{(Eq. 1)}$$

where ALT <3 × ULN is referred to as 'abnormality of liver test'.

- Cholestasis (equation 2):

$$\text{AP} \geq 2 \times \text{ULN} \textbf{ and } \frac{(\text{ALT/ULN})}{(\text{AP/ULN})} \leq 2 \quad \text{(Eq. 2)}$$

where AP <2 × ULN is referred to as 'abnormality of liver test'.

- Mixed (equation 3):

$$\text{ALT} \geq 3 \times \text{ULN} \textbf{ and } \text{AP} \geq 2 \times \text{ULN} \textbf{ with } \frac{(\text{ALT/ULN})}{(\text{AP/ULN})} > 2 \text{ to} < 5 \quad \text{(Eq. 3)}$$

The hepatitis pattern indicates underlying hepatocellular injury and may present either in the absence of symptoms, with malaise and abdominal pain, with jaundice or with acute liver failure. Generally, hepatocellular injury and the resultant elevation of transaminase levels tend to resolve over the course of several weeks after discontinuation of the offending drug. A corollary to this observation is that sometimes asymptomatic liver test abnormalities resolve despite continued drug use, a phenomenon referred to as adaptation. It appears that the subgroup that progresses to overt liver disease might do so because of a failure to adapt. At present, the mechanistic basis for adaptation remains elusive. Except in the case of paracetamol toxicity, the degree of ALT elevation is usually not reflective of the severity of disease. In fact, histological evidence of injury is often more severe than is suggested by clinical signs or laboratory studies.

The one index predictive of mortality in the context of acute drug-induced hepatitis is the presence of jaundice. Drug-induced hepatitis accompanied by a bilirubin level >3 × ULN, in the absence of biliary obstruction and Gilbert's syndrome, is associated

Table III. Autoantibodies in drug-induced liver disease

Autoantibody target	Drug
CYP2C9	Tienilic acid
CYP1A2	Dihydralazine
CYP3A	Antiepileptic drugs
CYP2E1	Halothane
mEH	Germander

CYP = cytochrome P450; **mEH** = microsomal epoxide hydrolase.

with a mortality of approximately 10% (range 5–50%).[21] Known as 'Hy's Law' in honour of the pioneering work of Hyman Zimmerman, this observation has been validated by two recent studies[22,23] and has been adopted by the FDA as a predictor of severe toxicity during clinical trials. In the interest of patient safety, the FDA has lowered the clinical trial threshold of hyperbilirubinaemia to 2 × ULN.[24]

The cholestatic pattern can be caused by canalicular cholestasis or ductular injury. Patients may present either with acute abdominal pain and fever, resembling acute biliary obstruction, or with chronic jaundice and pruritus. Although not usually as life-threatening as hepatocellular injury, cholestatic drug-induced liver injury may lead to chronic ductopenia and rarely cirrhosis.[25,26] Zimmerman[27] noted a fatality of <1% for cholestatic drug-induced liver injury, whereas a recent study has reported the more alarming mortality rate of 7.8%.[22] With cholestatic injury, liver test abnormalities tend to resolve over the course of several months. Presumably, the regeneration of cholangiocytes progresses more slowly than that of hepatocytes.

In a mixed pattern drug-induced liver injury, patients present with a combination of acute hepatitis and cholestasis. Fatalities have been reported in association with mixed hepatic injury with an incidence lower than that seen with either hepatocellular or cholestatic drug-induced liver injury.[22] Drugs that result in a cholestatic pattern may result in a mixed pattern and vice-versa. In clinical practice, it is therefore useful to group together the drugs that cause these two patterns. Table I lists the medications associated with each pattern of liver test abnormality.

Of the three patterns of liver injury, hepatitis is more commonly accompanied by acute liver failure. The latter is heralded by the combination of a prolonged prothrombin time (international normalised ratio ≥1.5) and any degree of mental alteration. This combination of coagulopathy and encephalopathy occurring <26 weeks after the onset of illness in a patient without pre-existing cirrhosis portends a grim prognosis in the absence of liver transplantation.[28]

Table IV. Selected non-drug causes of hepatic dysfunction

Aetiologies	Commonly ordered diagnostic tests
Alcohol	Transaminases (<300 U/L with AST : ALT >2 : 1)
Autoimmune disorders	
autoimmune hepatitis	Type 1: anti-smooth muscle Ab, antinuclear Ab
	Type 2: anti-liver kidney microsomal Ab
primary biliary cirrhosis	Anti-mitochondrial Ab
Biliary disorders	Ultrasound, ERCP, MRCP
Haemodynamic disorders	
Budd-Chiari syndrome	Doppler ultrasound
heart failure	Echocardiogram
ischaemia/hypoxia	Aminotransferases >1000 U/L, elevated lactate dehydrogenase level
portal vein thrombosis	Doppler ultrasound
veno-occlusive disease	Liver biopsy
Metabolic/genetic disorders	
α-1 antitrypsin deficiency	Absence of α-1 globulin on serum electrophoresis, diminished serum level of α-1 antitrypsin
Haemochromatosis	Elevated iron saturation, elevated ferritin level
Wilson's disease	Elevated serum and urinary copper levels; diminished serum ceruloplasmin
sepsis-induced cholestasis	Blood cultures
Viral hepatitis	
hepatitis A	Hepatitis A IgM Ab
hepatitis B	Hepatitis B surface antigen
	Hepatitis B core IgM Ab
hepatitis C	Hepatitis C Ab
	Hepatitis C qualitative RNA PCR

Ab = antibody; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **ERCP** = endoscopic retrograde cholangiopancreatography; **MRCP** = magnetic resonance cholangiopancreatography; **PCR** = polymerase chain reaction; **RNA** = ribonucleic acid.

Table V. Non-hepatic causes of abnormal liver tests

Test	Trend	Non-hepatic causes
Albumin	Depressed	Congestive heart failure
		Inflammatory states
		Malnutrition
		Nephrotic syndrome
		Protein-losing enteropathy
Alkaline phosphatase	Elevated	Malignancy
		Osseous disease
		Pregnancy
AST	Elevated	Myocardial infarction[a]
		Muscular disorders[a]
Bilirubin	Elevated	
	Direct	Dubin-Johnson syndrome
		Rotor's syndrome
	Indirect	Crigler-Najjar
		Gilbert's syndrome
		Haemolysis
		Ineffective erythropoiesis
Prothrombin time	Prolonged	Factor VII deficiency
		Vitamin K deficiency
		Warfarin use
		Acetylcysteine (large intravenous dose)

a May also result in elevation of ALT. In these cases, the AST to ALT ratio is usually ≥3 : 1.

ALT = alanine aminotransferase; **AST** = aspartate aminotransferase.

The jaundice seen with hepatocellular, cholestatic and mixed reactions results from direct hyperbilirubinaemia. However, a culprit medication should not be ruled out in instances of indirect hyperbilirubinaemia. Rifampicin (rifampin), probenecid and ribavirin diminish hepatic uptake of bilirubin, thereby inducing an elevation in the unconjugated bilirubin fraction.[29] Also, protease inhibitors, such as indinavir, inhibit bilirubin conjugation and can cause indirect hyperbilirubinaemia, especially in patients who are heterozygous or homozygous for the genetic defect of Gilbert's syndrome.[30]

2.2.2 Duration of Latency to Symptomatic Presentation

Hepatotoxicity can be further classified as predictable or unpredictable.[31] Predictable drug reactions occur with short latency (within a few days), with high incidence and in a dose-related fashion. Resulting from direct toxicity by the drug or its metabolites, the predictable reactions tend to be reproducible in animal models. Paracetamol is the principal drug in this category.[32] Conversely, unpredictable (idiosyncratic) reactions occur with variable, sometimes prolonged latency (1 week to 1 year), with low incidence and may or may not be dose-related.

The overwhelming majority of hepatotoxic drugs are associated with idiosyncratic reactions. These reactions tend to occur on a background of asymptomatic, usually transient liver injury that is detected as a disturbance in liver chemistries, particularly ALT.[33] This transient background disturbance is not necessarily predictive of the patient's vulnerability to serious further injury. In clinical trials, an ALT level >3 × ULN has been somewhat arbitrarily identified as a sensitive, but not necessarily specific, signal of liver toxicity.[16,34] The protracted latency of unpredictable reactions can be of intermediate duration (1–8 weeks), as seen with most immune-mediated reactions, or of long duration (up to 1 year), as seen with non-immune-mediated reactions and selectively few immune-mediated reactions (such as those induced by nitrofurantoin). When eliciting a drug history, it is insufficient to inquire solely about the current medical regimen. Drugs that result in immune-mediated reactions such as sulfonamides, erythromycin and amoxicillin/clavulanic acid may result in drug-induced liver injury 3–4 weeks following the termination of therapy.

2.2.3 Immune- and Non-Immune-Mediated Idiosyncratic Reactions

An immune-mediated idiosyncratic reaction is characterised by the presence of fever, rash, eosinophilia and autoantibodies (such as antinuclear and smooth muscle antibodies). However, these signs are not found invariably in conjunction with one another. An additional hallmark of immune-mediated reactions is the onset of a more rapid and severe relapse when the drug is reintroduced after a temporary interruption of treatment.[35] Although a rapid positive re-challenge is the most definitive signal of an immune-mediated reaction, it is evident on ethi-

cal grounds that demonstrating this clinical sign is rarely, if ever, indicated. In contradistinction, non-immune-mediated reactions are characterised by the absence of the aforementioned signs. Both immune- and non-immune-mediated reactions may be of either the hepatocellular or cholestatic/mixed type (table I). Although the recognition of features of hypersensitivity is useful clinically in the identification of a culprit drug, the absence of these features does not necessarily exclude an allergic mechanism as the cause of the toxicity.

2.2.4 Response to Drug Withdrawal

In general, resolution of liver test abnormalities following drug withdrawal (de-challenge) may be viewed as consistent with drug-induced liver injury. There are three important qualifiers to this statement: (i) following drug discontinuation, liver injury may sometimes worsen for weeks before improvement is seen; (ii) resolution may be delayed up to 1 year following de-challenge in the case of cholestatic reactions; and (iii) down-trending transaminase levels may portend hepatic failure rather than resolution of injury. In the latter instance, decreasing AST and ALT levels are reflective of the limited hepatic reserve that follows overwhelming hepatocellular death.

3. Risk Factors

Susceptibility to drug-induced liver injury is influenced by an interplay between age, sex, concurrent drug therapy, environmental factors, underlying disease states and genetic predisposition. In general, advanced age increases the risk for developing drug-induced liver injury.[1] For instance, being >49 years of age is a risk factor for isoniazid-induced hepatotoxicity.[36] Valproate sodium and erythromycin estolate are exceptional in that they predominantly induce hepatotoxicity in children.[37] In many instances, women have shown greater susceptibility to drug-induced liver injury. The Acute Liver Failure Study Group has demonstrated a female preponderance in acute liver failure due to both paracetamol (74%) and idiosyncratic drug reactions (67%).[38] Combination therapy in certain instances augments the risk of drug-induced liver injury. For example, concurrent use of isoniazid and rifampicin has more hepatotoxic potential than either therapy alone.[39] Underlying disease states, such as infection with human immunodeficiency, hepatitis B or hepatitis C viruses, increase the toxic potential of certain drugs, such as isoniazid.[40-42]

Environmental risk factors, such as ethanol use and malnutrition, deplete glutathione stores and potentiate paracetamol-induced toxicity.[43,44] Although there have been attempts to challenge the concept of an alcohol-paracetamol interaction (reviewed by Bromer and Black[45] and Rumack[46]), the FDA maintains a warning regarding paracetamol use for those who consume three or more alcoholic drinks per day.

Genetic variability may be the greatest determinant of susceptibility to drug-induced liver injury.[47] For instance, *N*-acetyltransferase 2[48] and CYP2E1[49] genetic polymorphisms are associated with susceptibility to antituberculous drug-induced hepatotoxicity. Few other well documented examples of genetic predisposition have been identified: glutathione *S*-transferase polymorphisms have been associated with troglitazone[50] and tacrine[51] hepatotoxicity; cytokine polymorphisms (interleukins 4 and 10) have been associated with diclofenac toxicity,[52] and human leucocyte antigen (HLA) associations have been described with cholestatic and mixed hepatocellular/cholestatic reactions.[53] Table VI lists examples of drugs and their associated risk factors.

Table VI. Examples of risk factors for drug-induced liver injury

Drug	Risk factors
Paracetamol (acetaminophen)	Chronic alcohol use, fasting, isoniazid use
Diclofenac	Female sex, osteoarthritis
Erythromycin	Young age
Halothane	Obesity
Isoniazid	HBV, HCV, HIV, alcohol use, older age, female sex, rifampicin (rifampin) use
Methotrexate	Chronic alcohol use, obesity, diabetes mellitus, chronic hepatitis, psoriasis
Valproate sodium	Young age, antiepileptic drug use

HBV = hepatitis B virus; **HCV** = hepatitis C virus.

4. Causality Assessment

A formal causality assessment tool was conceived in 1990 by eight renowned hepatologists from Europe and the US at the behest of the CIOMS. The opinion-based Roussel-Uclaf causality assessment method (RUCAM) is a scoring system that is predictive of the likelihood of drug-induced liver injury.[54] The system comprises seven weighted criteria that are tabulated and taken into consideration in a graded fashion along with the type of liver injury (hepatocellular or cholestatic/mixed). The criteria include time to onset of hepatic injury following initial drug exposure, course of the reaction, presence of risk factors, concomitant drug exposure, exclusion of non-drug causes of liver injury, previous information regarding the known hepatotoxic potential of the drug and response to re-challenge. Depending on whether the reaction is hepatocellular or cholestatic/mixed, the RUCAM system incorporates different markers for four of the criteria: time to onset, course, risk factors and response to re-challenge.

An additional three points may be assigned if a known toxic serological level exists and is measured. The scoring system also makes allowance for future advancements in the detection of drug-induced liver injury. If a specific diagnostic test for drug-induced liver injury were available and positive, three extra points could be assigned; if the test were negative, three points could be deducted, and if no test were available (the current case for all drugs) then no points would be assigned. On the basis of the tabulated score, the likelihood of drug-induced liver injury is categorised as highly probable (>8), probable (6–8), possible (3–5), unlikely (1–2) or excluded (≤0). The RUCAM system has been validated using drug-induced liver cases with positive re-challenge[55] and it appears to be superior to other causality assessment systems in head-to-head comparison.[56] However, there is room for improvement. For example, pregnancy, age >55 years and alcohol use are risk factors for a very limited repertoire of drugs. Furthermore, at the time of initial presentation, there is no information regarding the course of the drug toxicity. Lastly, although based on expert opinion, the grading for each category remains arbitrary.

5. Prevention

5.1 Signals of Toxicity in Clinical Trials

Acute liver failure, although sometimes encountered in clinical trials, is unlikely to occur in a population of several thousand clinical trial patients because the incidence of acute liver failure is in the range of 1 in ≤10 000.[57] It is therefore necessary to look at other milestones. Nearly all recent examples of allergic and non-allergic idiosyncratic hepatotoxicity have been accompanied by an increased frequency of ALT abnormalities in clinical trials. An ALT elevation of 3 × ULN represents very mild injury and may be seen in placebo-treated patients. An ALT elevation of >3 × ULN is seen with low incidence (0.2–1%) in placebo-treated patients. On the other hand, a statistically significant doubling (or more) in the incidence of serum ALT elevation >3 × ULN is nearly universally described with idiosyncratic hepatotoxins. Therefore, an ALT level of >3 × ULN has been identified as a sensitive signal for liver toxicity. However, this threshold is not specific and is thus not necessarily predictive of more severe outcomes. For example, although most statins are associated with a dose-related increase in the incidence of ALT elevation of >3 × ULN, acute liver failure occurs in one in a million treated patients, an incidence no greater than that estimated for idiopathic acute liver failure.[58] More specific predictors of liver failure are an ALT elevation of >8–10 × ULN (very rarely seen in placebo-treated patients) and an ALT level of >3 × ULN accompanied by a bilirubin level of >2 × ULN (modified Hy's Law, as described in section 2.2.1).[59]

When milestones predictive of hepatotoxicity are met in clinical trials, a benefit-risk analysis will favour either approval, approval for restricted use or withdrawal. The benefit-risk analysis usually makes allowance for the following considerations: the target illness (e.g. a severe illness such as cancer may justify the risk of adverse events); the duration of drug use (e.g. a medication for diabetes mellitus

would need to be safe for long-term use); the safety and efficacy of existing medications for the same indication; and the likely efficacy of a monitoring programme to minimise serious adverse events.

The experience with troglitazone is illustrative of the process of benefit-risk analysis. During clinical trials, the incidence of ALT level >3 × ULN in treated patients was 1.9% as compared with 0.6% in patients receiving placebo.[60] An ALT level of >10 × ULN was noted in 0.6% of those who received troglitazone and none of the controls. Roughly 1 in 1000 patients fulfilled the criteria of Hy's Law. There was no instance of acute liver failure.[60] In the postmarketing experience with about 2 million treated patients, nearly 100 cases of acute liver failure were reported to the FDA (1 in 20 000 patients taking troglitazone). At first, it was decided that monthly monitoring would likely protect users and the drug was kept in the marketplace. As described in section 5.2, monitoring proved an ineffective safeguard. Several new drugs in the same class were shortly thereafter approved, and following a number of months of postmarketing experience, it was concluded that these agents were probably less likely to induce severe hepatotoxicity, leading to the withdrawal of troglitazone.

Recently, the thrombin inhibitor ximelagatran showed an even more significant signal in clinical trials, with an incidence of ALT level >3 × ULN in 7.9%, ALT level >10 × ULN in 1.9%, and an elevated ALT level with bilirubin >2-fold in 0.5% of patients, respectively.[61] Although adjudication of the latter cases (which met criteria for the modified Hy's Law) decreased their incidence to closer to 0.1%, there were several cases of acute liver failure.[61] The drug was not approved in the US and, as a result of its hepatotoxicity, was voluntarily withdrawn from the market in Europe following short-term use.

5.2 Monitoring in Clinical Practice

As mentioned previously, there exists a background incidence of drug-induced, mild, reversible liver injury, which comes to medical attention only as a result of serological testing. From this background of mild injury, a minority of individuals will emerge with overt disease. An approach to averting serious liver injury would therefore couple surveillance for minor injury (monthly monitoring appears most practical), and early drug cessation when minor injury is detected.

Although rational, this approach has multiple fallacies and pitfalls. First, this approach would only be applicable to the non-immune-mediated idiosyncratic reactions since these are delayed. Drugs that result in predictable reactions do not warrant screening, since the reactions they induce occur early and in a dose-dependent fashion. There also seems to be little justification for screening in the immune-mediated idiosyncratic group, since the reactions occur relatively early, usually progress very rapidly to become symptomatic and are therefore easily recognised. Secondly, since only a minority of patients will develop overt disease, drug cessation with mild reversible injury will unnecessarily deny patients a potentially important therapy. Thirdly, compliance with routine hepatic serological surveillance has proven to be poor. One may conceivably address this compliance issue through risk-management programmes that limit prescription refills in accordance with the results of serum ALT monitoring. Fourthly, the rate of development of overt disease needs to be gradual for routine monitoring to be effective in preventing life-threatening disease. Unfortunately, severe reactions are known to occur precipitously following months of therapy, despite persistently normal screening tests. The importance of this final caveat was highlighted by the troglitazone experience. Of 12 cases of acute liver failure from troglitazone, in whom monthly monitoring was actually performed, the liver injury progressed from normal ALT to acute liver failure within 1 month in nine cases.[62]

Thus, where serological monitoring is concerned, we are left with a conundrum. On the one hand, we have unconvincing efficacy, poor compliance and far more patients withdrawn from treatment than would actually experience a serious adverse reaction. On the other hand, in cases where a benefit-risk analysis would favour continued therapy, monthly

monitoring may have some benefit compared with no monitoring at all.[34] However, serological monitoring does not obviate the need for patient education regarding the symptoms of hepatotoxicity. Beyond being vigilant about those symptoms more specific to hepatic disease, such as jaundice and pruritus, patients should be instructed to bring to medical attention symptoms of nausea, malaise, anorexia and fatigue. Indeed, in the case of isoniazid chemoprophylaxis, reporting of symptoms at monthly visits proved effective in averting serious consequences without the need for ALT measurements.[63] However, it should be noted that the population in this study was relatively young (<35 years of age); thus, the risk was not as great as in an older population.

6. Management and Prognosis

Therapeutic interventions in drug-induced liver injury cases remain principally limited to the cessation of the offending drug, supportive care and monitoring for acute hepatic failure. These three measures aside, the physician's armamentarium consists of only two antidotes: acetylcysteine for paracetamol toxicity[28] and intravenous carnitine for cases of valproate sodium overdose.[64] Glucocorticoids for immune-mediated reactions[65] and ursodeoxycholic acid for cholestatic drug-induced liver injury[66] remain controversial therapies.[67]

Serial biochemical assessment in conjunction with consultation from a hepatologist is warranted in cases of suspected drug-induced liver injury. With the onset of acute liver failure, early transfer to a transplantation centre is imperative.

Overall survival is better for paracetamol-induced reactions than for idiosyncratic cases, with spontaneous survival rates of 62% and 26%, respectively.[38] Furthermore, transplant-free survival rate and rate of liver transplantation are similar between suicidal and unintentional paracetamol-induced acute liver failure groups.[4]

7. Pathogenesis

In general, toxicity of drugs involves the obligate participation of reactive metabolites.[68] Exposure to these intermediates is determined by genetic and environmental influences of phase 1 (CYP), phase 2 (detoxification) and phase 3 (excretion/transport). Exposure to some critical level of toxic metabolite has important chemical consequences, including covalent binding, oxidative stress and metabolic disturbances, which may be overwhelming or which may activate signal transduction, and/or gene expression, which participate in injury.[1,33] The subject of pathogenesis has been reviewed elsewhere.[34] Hepatocellular death mechanisms are aspects of drug-induced liver injury pathogenesis requiring more attention and, therefore, form the focus of the current discussion.

7.1 Pathogenesis of Drug-Induced Hepatitis

Hepatocellular death underlies the clinical manifestations of drug-induced hepatitis. Cellular demise may occur by means of either apoptosis or necrosis. The former is a programmed process of cellular disassembly that necessitates the use of adenosine triphosphate (ATP)[69] and that involves cellular shrinkage and fragmentation into discrete bodies that maintain intact plasma membranes (apoptotic bodies). The apoptotic bodies are rapidly cleared by means of phagocytosis, thereby leaving little substrate for an inflammatory response. On the other hand, necrosis involves a profound loss of mitochondrial function with resultant ATP depletion and loss of ion homeostasis that leads to cellular swelling and lysis. The lytic release of cellular contents promotes a local inflammatory reaction. Loss of membrane integrity is an essential characteristic distinguishing apoptosis from necrosis.[70] In certain cases, massive apoptosis may overwhelm the phagocytic faculties of the liver, thereby resulting in substantial inflammation and secondary necrosis.

7.1.1 Apoptotic Cell Death

The disassembly process in apoptosis is executed by caspases (cysteine-dependent aspartate-specific proteases), a group of proteolytic enzymes that

cleave protein chains after aspartic acid residues.[71] These enzymes exist as zymogens, whose activation occurs either by self-cleavage or cleavage by other caspases. The cascade of caspase activation is instigated by initiators (caspases 8 and 9) and terminates with executioners (caspases 3, 6 and 7) that carry out the actual cellular disassembly. In some cell types, initiator-induced activation of executioner caspases is sufficient to promote apoptosis. However, in hepatocytes, the activation of executioner caspases requires an amplification mechanism. The central participant in this amplification mechanism is the mitochondrion, which releases caspase-activating proteins from its intermembrane space into the cytoplasm.[72] Release of these intermembrane proteins (cytochrome c, Smac, apoptosis inducing factor) requires an increase in the permeability of the mitochondrial outer membrane. The degree of mitochondrial membrane permeability is in turn predicated on the balance between the proapoptotic and antiapoptotic influences of the B cell lymphoma-2 (Bcl_2) family members.[73,74] The Bcl_2 family is a group of proteins with domains (BH1 to BH4) homologous to those of the Bcl-2 oncogene, and whose function is to regulate mitochondrial integrity in response to cellular stress. The prosurvival members (Bcl-2 and Bcl-X_L) possess all four homology domains. The proapoptotic members are divisible into two subgroups: (i) the Bax subfamily (includes Bax and Bak) whose members possess the BH1, BH2 and BH3 domains; and (ii) the BH3 only proteins (Bid, Bim, Bmf), which possess only the BH3 motif. The stimulus that tilts Bcl_2 influence in the proapoptotic direction and hence augments mitochondrial permeability may arise either at the cellular surface (extrinsic pathway) or within the cell itself (intrinsic pathway).

Extrinsic Pathway

The extrinsic pathway begins with the engagement of a death receptor at the cell surface. The principal hepatic death receptors are tumour necrosis factor receptor-1 (TNF-R1) and Fas, which are activated when bound by tumour necrosis factor (TNF) and Fas ligand (FasL), respectively.[75,76] Once the death receptor is engaged, its cytoplasmic domain binds adaptor proteins (TRADD, FADD), which then recruit procaspase 8. Once recruited, procaspase 8 self-cleaves to release caspase 8. Caspase 8 cleaves Bid, thereby activating it to induce Bax and Bak translocation into the mitochondrion. Upon translocating, Bax and Bak promote mitochondrial permeabilisation, and the release of cytochrome c, Smac and apoptosis inducing factor.[73] Drug-induced activation of the extrinsic pathway is mediated by the immune system. TNF-R1 and Fas are activated by the innate and adaptive immune systems, respectively.

Innate Immune System Activation of TNF-R1 and Other Effects

TNF is released by first-line defence agents of the innate immune system such as Kupffer and natural killer (NK) cells and is a known mediator of toxin-induced hepatotoxicity. For instance, neutralisation of TNF[77] or use of TNF-R1 knock-out mice[78] prevents carbon tetrachloride hepatoxicity. Drugs and their metabolites may either induce an increase in TNF production or sensitise hepatocytes to the effects of TNF. The former mechanism has been noted in the case of paracetamol.[79,80] Although TNF knock-out is not protective of paracetamol toxicity,[81] knock-out of a TNF downregulator, C-C chemokine receptor 2 (CCR2), results in increased TNF production and toxicity in response to paracetamol.[82] Paracetamol-induced toxicity in CCR2 knock-out mice is abrogated by immunoneutralisation of TNF.[82]

There are two known mechanisms by which hepatocytes can become sensitised to the effects of TNF. The first involves inhibition of nuclear factor-κB (NF-κB), and the second involves glutathione (GSH) depletion. NF-κB is activated by TNF-R1 and works as a downstream stimulator of survival gene transcription. Hence, engagement of TNF-R1 activates two competing signal transduction cascades: a proapoptotic cascade via caspase 8 and a prosurvival cascade via NF-κB.[83] Actinomycin D and certain toxins such as galactosamine and alpha-amanitin (mushroom poison) inhibit the NF-κB-mediated transcription of survival genes, thereby shifting the balance in favour of the TNF-R1-medi-

ated proapoptotic cascade.[84] GSH depletion sensitises hepatocytes to TNF by rendering the hepatic mitochondrion incapable of detoxifying the TNF-induced burden of reactive oxygen species.[85,86] Furthermore, the known inhibition of NF-κB-dependent transcription by GSH depletion[87] may play a role in hepatocyte sensitisation to TNF.

The innate immune system also participates in paracetamol-induced toxicity by means that are independent of TNF-R1 activation. These mechanisms involve the recruitment of NK/natural killer T (NKT) cells and neutrophils. NK and NKT cells recognise the antigens of aberrant cells in the context of CD1d, and respond by killing these cells or by activating other components of the immune system via chemokines and cytokines. NK/NKT cells comprise a substantial portion of the hepatic leucocyte population (20–50%)[88-91] and have been implicated in the pathogenesis of disorders such as viral[92] and autoimmune hepatitis.[93] Recently, NK/NKT cells have been shown to play a critical role in paracetamol-induced hepatotoxicity. Monocloncal antibody depletion of NK/NKT cells significantly protected mice from paracetamol-induced liver injury, as evidenced by a decreased serum ALT level, decreased hepatic necrosis, improved mouse survival, inhibition in the expression of interferon-γ (IFNγ), FasL and chemokines, as well as decreased neutrophil accumulation in the liver.[94] IFNγ is a proinflammatory cytokine that acts as an important mediator of NK/NKT-induced necrosis. NK/NKT cells are the source of IFNγ in paracetamol-challenged mice[94] and IFNγ-null mice are protected against paracetamol.[95] Osteopontin is another inflammatory mediator that is mainly produced by NK/NKT cells.[96-98] Paracetamol-induced liver injury is associated with an increased expression of osteopontin, and osteopontin-knockout mice are less susceptible to paracetamol hepatotoxicity than wild-type mice.[99]

With respect to neutrophils, there is an increase in their number within the liver of paracetamol-treated animals.[94,95,100,101] As mentioned previously, protection against paracetamol-induced hepatotoxicity by NK/NKT depletion is associated with reduced hepatic neutrophil accumulation. Neutrophil-depleted mice demonstrate significantly reduced liver injury and improved survival.[102,103] Knockout of CXC chemokine receptor 2 (a receptor critical for neutrophil migration) reduces neutrophil migration and protects against paracetamol-induced injury.[103] Both neutrophil depletion and CXC chemokine receptor 2 knockout result in reduced inducible nitric oxide synthase (iNOS) production in the liver.[103] Intracellular adhesion molecule (ICAM)-1 expression is increased in paracetamol-treated mice,[95] and when it is deficient, is associated with a resistance to paracetamol-induced injury and a reduction in hepatic neutrophil accumulation.[102] However, there remains controversy as to whether neutrophils directly contribute to the liver injury process or are recruited to the liver for the removal of cell debris.[104]

Adaptive Immune System Activation of Fas

Generally, low-molecular weight organic chemicals or drug metabolites are not immunogenic. However, they may act as haptens and covalently bind hepatic proteins, such as CYP enzymes, thereby altering their conformation (haptenisation). The innate immune system is sensitised to these altered proteins through the action of macrophages. The latter scavenge senescent hepatocytes and process the haptenised proteins into peptides that are presented on the macrophage surface bound to the major histocompatibility complex (MHC) class II. Cell cluster differentiation (CD)-4 T-cells then recognise the peptide/MHC-II complex, become primed, undergo clonal expansion and are enabled to provide cytotoxic CD8 cells with the requisite co-stimulatory signals. The haptenised hepatic proteins can then trigger apoptosis via the now sensitised adaptive immune system when they are processed into cytosolic peptides and presented at the hepatocyte surface by MHC class I.[105-107] Cytotoxic CD8 Tcells bear the FasL and once they recognise the foreign peptide/MHC-I complex, they induce apoptosis of the hepatocyte by engaging its Fas receptor. Haptenisation alone may be insufficient to trigger an immune reaction, or could induce a non-pathogenic immune response (e.g. the common oc-

currence of anti-CYP2E1 antibodies in halothane exposed anaesthesiologists).[108] For the development of an immune response, it has been proposed that a co-stimulatory trigger, a so-called 'danger' signal is required.[109] The danger signal may come in the form of mild background hepatic injury (as discussed previously) or a concomitant infectious or inflammatory condition. For instance, immune-mediated hepatotoxicity is more common in patients who have AIDS.[110]

Intrinsic Pathway

Drug-induced activation of the intrinsic pathway is independent of the immune system, and results from direct damage to cellular organelles by the drugs or their metabolites. Cytoskeletal stress releases Bmf, and microtubular stress releases Bim. Bmf and Bim in turn translocate to the mitochondrion where they promote outer membrane permeabilisation.[74] Other organelles such as the endoplasmic reticulum and the nucleus also respond to stress by promoting mitochondrial permeability. One important stress responder is Jun N-terminal kinase (Jnk); sustained activation of Jnk in response to oxidative stress or inhibition of NF-κB promotes activation of proapoptotic Bcl_2 family members in various contexts.[111]

Effect of Mitochondrial Permeabilisation

Once the proapoptotic members of the Bcl_2 family are activated by either the extrinsic or the intrinsic pathway, they induce mitochondrial outer membrane permeabilisation (MOMP) and the release of cytochrome c, Smac and apoptosis inducing factor. Cytochrome c, in conjunction with ATP, binds apoptotic protease activating factor 1 (Apaf-1) thereby permitting the latter to adopt the conformation (apoptosome) required for caspase 9 activation.[112] Caspase 9 then activates the executer caspase 3. Smac promotes apoptosis by countering the inhibitory effect that inhibitors of apoptosis proteins (IAPs) have on caspases.[113] Apoptosis inducing factor activates DNA fragmentation.[114,115]

7.1.2 Necrotic Cell Death

Whether drug-induced hepatocyte death occurs via apoptosis or necrosis appears to depend on whether or not the cell is sensitised to the immune system and on the severity of the oxidative stress. Sensitisation to the immune system results in apoptosis via the extrinsic pathway. Moderate oxidative stress results in apoptosis via the intrinsic pathway. Severe oxidative stress results in profound loss of mitochondrial function with resultant ATP depletion, loss of ion homeostasis and necrotic cell lysis. In the context of severe oxidative stress, the apoptotic pathway is furthermore inhibited: caspases are inhibited by severe oxidative stress and, as mentioned in the previous section, ATP is required for the formation of the apoptosome. Nevertheless, components of the apoptosis programme, which act upstream of the mitochondrion, may participate in necrotic cell death. In this regard, an important recent example is the role of Jnk in paracetamol-induced necrosis.[111]

A key feature of necrosis is the participation of the mitochondrial permeability transition (mPT), a pore within the inner mitochondrial membrane. Composed of a number of proteins including adenine nucleotide translocase and cyclophilin D, mPT opens in response to reactive oxygen species and calcium. Opening of the mPT leads to the collapse of mitochondrial function, a key step in the development of necrosis in paracetamol-induced injury.[116]

The pathogenesis of hepatocellular injury is outlined in figure 1.

7.2 Pathogenesis of Drug-Induced Cholestasis

Drug-induced cholestasis may be bland (histological analysis reveals no inflammation or necrosis) or accompanied by bile duct injury and inflammation. The former is illustrated by drugs that inhibit the bile salt excretory protein (BSEP), such as rifampicin, ciclosporin (cyclosporine) and glibenclamide.[117] Although sulindac,[118] bosentan[119] and troglitazone[120] also inhibit BSEP, it is likely that severe toxicity with these drugs is due to other mechanisms. However, the bulk of clinically overt cholestatic and mixed hepatitis/cholestasis reactions seem to be associated with bile ductular injury and inflammation. In these circumstances, it is hy-

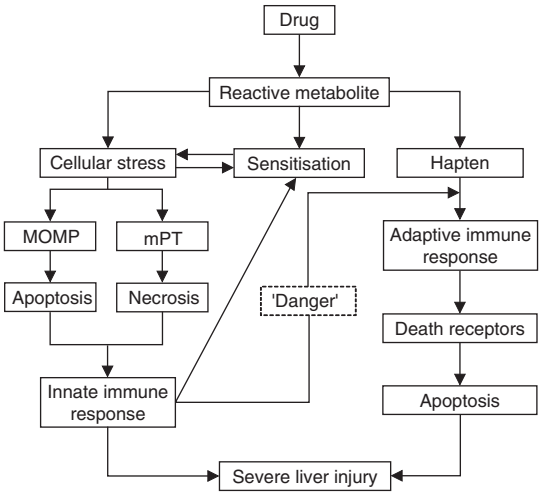

Fig. 1. Hypothesis for the pathogenesis of drug-induced hepatocellular injury. The reactive metabolite of a drug induces cellular stress, which results in either mitochondrial outer membrane permeabilisation (MOMP) or the formation of a pore in the mitochondrial inner membrane termed mitochondrial permeability transition (mPT). The permeabilisation of the mitochondrion results in cell death whereby MOMP formation leads to apoptosis and mPT formation induces necrosis. Cell death in turn leads to the recruitment of members of the innate immune system such as natural killer cells, natural killer T cells and neutrophils. Participation of the innate immune system augments cellular death and results in severe liver injury. The reactive metabolite of a drug may also act as a hapten and stimulate the adaptive immune response. A co-stimulatory trigger ('danger signal') in the form of background inflammation is required for the activation of the innate immune system. The activated innate immune system results in the engagement of death receptors and apoptosis, thereby contributing to severe liver injury. A destructive cycle is set in motion whereby reactive metabolites, cellular stress and the innate immune system sensitise the hepatocytes to further injury.

pothesised that the drug metabolite undergoes canalicular excretion, thereby exposing the bile ductular cells to the direct toxic effects or the immune-sensitising effects of the metabolite. Ductular excretion of some drug metabolites appears to be mediated by multidrug resistance-associated protein-2 (MRP2). This concept is illustrated by the abrogation of α-naphthylisothiocyanate-induced cholestatic disease in MRP2-deficient rats.[121] Cholestatic disease related to the use of flucloxacillin[122] and terbinafine[123] appears to result form canalicular excretion of toxic metabolites.

8. Conclusions

As a result of its protean manifestations, drug-induced liver injury must be included in the differential diagnosis of all patients with hepatobiliary derangements. The diagnosis of drug-induced liver injury is predicated on the exclusion of other plausible aetiologies and on the identification of a clinical signature that consists of the pattern of liver test abnormality, the duration of latency to symptomatic presentation, the presence or absence of immune-mediated hypersensitivity and the response to drug withdrawal. A causality assessment must also include the patient's susceptibility profile. In these respects, the RUCAM scoring system remains a useful, although limited, adjunct in the identification of culprit drugs.

Protection of the public from potentially hepatotoxic medications begins with the detection of toxicity signals in clinical trials that are then weighed against the therapeutic benefits of the novel agent. For those hepatotoxic drugs that make it to the market, monthly monitoring of serum ALT levels may permit the early detection of those who could experience more serious injury with continued use. Those who do experience more serious injury can usually be afforded only supportive care, with early referral to a transplantation centre once signs of acute liver failure become manifest. Drug-induced hepatitis results from hepatocellular apoptosis or necrosis, whereas drug-induced cholestasis occurs usually as a result of bile ductular injury. Elucidation of these mechanisms will allow for effective therapies and for patient-tailored preventive measures. At present, the principal preventive measure is the physician's judicious dispensing of drugs.

According to William Osler, "the desire to take medicine is perhaps the greatest feature which distinguishes man from animals".[124] In certain instances, the patient's desire may direct the physician's prescriptive choices. An example is the use of antibacterials to placate patients affected by viral upper respiratory infections. Given the fallibility of the signals of liver toxicity in clinical trials and the limitations of available therapeutics for drug-induced liver injury, an 'ounce of prevention' in the

form of patient education becomes imperative. For instance, patients may be informed about the recent case reports of severe hepatotoxicity that resulted from the use of telithromycin, a ketolide indicated for use in respiratory tract infections: one case required liver transplantation, and one case resulted in death.[125] Given the advantages afforded by modern medicines that were lacking in Osler's time, it would be irresponsible to adopt to the letter his suggestion that "one of the first duties of the physician is to educate the masses not to take medicine".[126] Dated though this statement may be, implicit within it is a counsel to caution that remains very relevant.

9. Future Considerations

The field of drug-induced liver injury faces a number of immediate challenges. On the preclinical level, it is critical to achieve a better understanding of mechanisms as well as the transcriptomic, proteomic and metabolomic signatures that will facilitate the prediction of human toxicity and may provide better biomarkers for application to diagnosis. At the clinical level, aside from diagnostic biomarkers and improved causality assessment methods, there is considerable promise in investigating genetic polymorphisms, which may predict susceptibility to drug-induced liver injury. This understanding of polymorphisms should further enlighten our understanding of pathophysiology and protect patients at risk, while permitting use in others. The gene targets for exploration include those that specifically relate to determinants of drug and toxic metabolite exposure, as well as those of general mechanistic potential (i.e. kinases, transcription factors), which respond to intracellular stress and the innate and adaptive immune systems.

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