Concept Paper

Premarketing Evaluation of Drug-Induced Liver Injury

Draft — Not for Implementation

This concept paper has been prepared by the Hepatotoxicity Working Group comprising staff from the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research. This concept paper should not be viewed as a regulation or guidance.

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1. INTRODUCTION

This concept paper is intended to stimulate discussion from the pharmaceutical industry and other investigators who are conducting new drug development about assessing the potential for a drug[1] to cause ***severe*** liver injury (i.e., fatal, or requiring liver transplantation). In particular, the concept paper addresses how laboratory measurements that signal the potential for such drug-induced liver injury (DILI) can be obtained and evaluated during drug development. This evaluation is important because most drugs that cause severe DILI do so infrequently; typical drug development databases with up to a few thousand subjects exposed to a new drug will not show any cases. Databases do, however, often show evidence of a drug's potential for severe DILI if clinical or laboratory data are properly evaluated for evidence of lesser injury that may or may not be serious (disabling, requiring or prolonging hospitalization, or potentially life-threatening), but may predict the ability to cause more severe injuries. This document describes an approach that can be used to distinguish signals of DILI that identify drugs likely to cause significant hepatotoxicity from signals that do not suggest such a potential. This document does not address issues of preclinical evaluation for potential DILI, nor the detection and assessment of DILI after drug approval and marketing.

2. HEPATOTOXICITY

2.1 BACKGROUND

Hepatotoxicity has been the most frequent single cause of drug marketing safety withdrawals for the past 50 years (iproniazid) and continuing to the present (ticrynafen, benoxaprofen, bromfenac, troglitazone). Hepatotoxicity discovered after approval for marketing also has limited the use of many drugs, including isoniazid, labetalol, trovafloxacin, tolcapone, felbamate, and nefazodone (Temple 2001). Several drugs have not been approved in the United States because European marketing experience revealed their hepatotoxicity (ibufenac, perhexiline, alpidem). Finally, some drugs were not approved in the United States because premarketing experience revealed marked hepatotoxicity (fialuridine) or provided enough evidence of potential toxicity to result in nonapproval (dilevalol, tasosartan, ximelagatran). Although most significant hepatotoxins have caused predominantly hepatocellular injury, signaled by leakage of aminotransferases (AT) from injured liver cells without prominent evidence of obstruction, the pattern of injury can vary. For example, perhexiline caused cirrhosis and fialuridine caused mitochondrial injury resulting in hepatic failure. Many drugs cause cholestasis, but in general this is reversible and does not lead to death or transplant; an exception is benoxaprofen, which

[1] This document uses the term *drug* or *product* to refer to all products, except blood products, regulated by the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research, including vaccines, and uses the term *approval* to refer to both drug approval and biologic licensure.

did cause fatalities (Taggert and Alderdice 1982). Recent experiences, and retrospective evaluation of earlier experiences, lead us to believe that appropriate premarketing testing and analysis may improve the early detection of drugs that can cause severe hepatocellular injury.

Drugs can cause many types of injury to the liver. These injuries resemble almost all known diseases of the liver and there are no pathognomonic findings, even upon liver biopsy, that make diagnosis of DILI certain. It is therefore essential to gather additional clinical and laboratory information, to observe the time course of the injury, and to seek alternative causes of the liver injury, such as acute viral hepatitis A, B, or C, autoimmune or alcoholic hepatitis, biliary tract disorders, and circulatory problems of hypotension or right heart congestive failure that may cause ischemic or hypoxic hepatopathy. It is also prudent to assess the subject for previously existing liver disease, such as smoldering chronic hepatitis C or nonalcoholic steatohepatitis, that may or may not have been recognized before starting exposure to the experimental drug.

Only the most overt hepatotoxins can be expected to show cases of severe DILI in the 1,000 to 3,000 subjects typically studied and described in a new drug application (NDA) or the smaller populations typical of a biologics license application (BLA). Overtly hepatotoxic agents (carbon tetrachloride, chloroform, methylene chloride) are toxic to anyone receiving a large enough dose, and drugs that cause such predictable and dose-related injury generally are discovered and rejected in preclinical testing. More difficult to detect is toxicity that is not predictable or clearly dose-related, but seems to depend on individual susceptibilities that have, to date, not been characterized. Most of the drugs withdrawn from the market for hepatotoxicity have had rates of death or transplantation in the range of 1 per 10,000 or less, so that a single case of such an event probably would not be found even if 5,000 to 10,000 subjects were studied. Cases of severe DILI have therefore rarely been seen in drug development programs of significantly hepatotoxic drugs.

What are regularly seen during development are mild liver injuries, often laboratory signals without any symptoms. The problem is that both drugs capable of severe DILI and drugs that have a low potential for causing severe injury (aspirin, tacrine, heparin, hydroxyl-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (*statins*)) generate these types of signals. An approach is therefore needed that can distinguish drugs likely to cause severe DILI from drugs unlikely to do so.

In general, the types of liver injury caused by drugs can be categorized as one of two types: predominantly hepatocellular injuries and predominantly cholestatic injuries, although mixed injuries also can occur, especially later in the course of injury. Hepatocellular injury is indicated by rises in serum AT activities reflecting release of alanine or aspartate aminotransferase (ALT or AST) from injured liver cells. The ability to cause some hepatocellular injury is not a reliable predictor of a drug's potential for severe DILI. Many drugs that cause transient rises in serum AT activity do not cause progressive or severe DILI, even if drug administration is continued. It is only those drugs that cause hepatocellular injury extensive enough to affect the liver's functional ability to clear bilirubin from the plasma or to synthesize prothrombin that cause severe DILI. It is important to identify those drugs as rapidly as possible.

Cholestatic injuries to hepatic bile duct cells are relatively common, but generally are more likely to be reversible after stopping the offending drug, and less likely to cause severe liver injury, although benoxaprofen was an exception.

Some drugs induce what seems to be an immunologic reaction, with systemic features such as fever, skin rash, and eosinophilia, but it has not been possible to identify a role of antibodies in liver injuries caused by such drugs.

The drugs that have caused severe DILI in humans have not shown clear hepatotoxicity in animals, generally have not shown dose-related toxicity, and, as noted, generally have caused low rates of severe injury in humans (1/5,000 to 1/10,000). These reactions thus appear to reflect host factors and individual susceptibility. Consequently, they have been termed "idiosyncratic," meaning dependent upon the individual person's particular constitution. Whether they are the result of genetic or acquired differences has not yet been established, and to date no genetic, metabolic, or other characteristic has been found to predict severe DILI in an individual.

Some severe DILI examples have been different from the more commonly seen hepatocellular idiosyncratic type. Perhexiline, an anti-anginal drug marketed in Europe, produced toxicity within months that had the histological appearance of alcoholic cirrhosis (Pessayre et al. 1979). Fialuridine caused modest acute liver injury, but most strikingly led to severe metabolic acidosis and multiorgan failure as mitochondrial oxidative capacity was obliterated over a period of months (Kleiner et al. 1997; Semino-Mora et al. 1997). Valproic acid may cause hyperammonemic encephalopathy even without notable rises in serum AT activities. Benoxaprofen (Oraflex) induced intrahepatic cholestasis that over many months led to significant, sometimes fatal, liver injury, especially in elderly patients (Taggart and Alderdice 1982).

Idiopathic hepatotoxins may cause liver injury evidenced by AT elevations in a substantial fraction of patients (about 20 percent for isoniazid), but most patients recover with or without cessation of treatment (a process sometimes called adaptation). For some drugs, however, a small fraction of the injured patients do not recover, and it is the drugs that cause this extent of injury that need to be identified and distinguished from other drugs that never, or rarely, cause severe injury, such as tacrine (as many as 50 percent of patients develop elevated serum ATs), aspirin, statins, or heparin, for which there have been very few reports of severe injury.

2.2 SIGNALS OF DILI AND "HY'S LAW"

As noted, although a typical NDA or BLA database will not usually show any cases of severe DILI, many drugs, including both significant hepatotoxins and drugs that do not cause severe liver injury, can cause laboratory evidence of hepatic injury, with leakage of liver enzymes and the appearance in blood of elevations in serum AT to levels of 3-, 5-, and greater times the upper limit of the normal reference range (ULN). The finding of a higher rate of such elevations in drug-treated subjects than in a control group is a sensitive signal indicating that the drug may have the potential to cause severe DILI and calls for close attention to the data. A more specific signal of such potential is a higher rate of more marked AT elevations (10x-, 20xULN), with cases of increases >1,000 U/L increased concern. The single clearest (most specific) predictor

found to date of a drug's potential for severe hepatotoxicity is evidence of reduced overall liver *function* in one or more patients, manifested by increased serum total bilirubin (TBL), not explained by any other cause, together with AT elevation in the study population.

Recognition of the importance of altered liver function, in addition to liver injury, began with the observation of the late Hyman J. Zimmerman (Zimmerman 1978) that drug-induced hepatocellular injury (transaminase elevation) accompanied by jaundice had a bad prognosis, with a 10 to 50 percent mortality. The reason for this now seems clear. The liver has a large excess of bilirubin-excreting capacity; injury to hepatocytes sufficient to cause jaundice or near jaundice (i.e., a bilirubin >2 mg/dL) represents an extent of damage so great that recovery may not be possible in some patients. Zimmerman did not specifically state how the determination of drug-induced injury was to be made, what activity of AT was needed to define hepatocellular injury, nor the specific level of serum bilirubin needed for jaundice to be noted (Zimmerman 1999), but his observation that hepatocellular injury sufficient to impair bilirubin excretion was ominous has been used at the Food and Drug Administration (FDA) over the years to identify drugs capable of causing severe liver injury, as distinct from drugs that cause lesser injury (AT elevation) but not severe injury (aspirin, tacrine, heparin). The observation of the critical importance of altered liver function has been referred to informally as "Hy's Law" (Temple 2001; Reuben 2004).

Briefly, a "Hy's Law" signal of the potential for severe drug-related hepatotoxicity has three components:

1. The drug causes hepatocellular injury, generally shown by more frequent 3-fold or greater elevations above the upper limit of normal (ULN) of ALT or AST than the control agent.
2. Among subjects showing such AT elevations, often with ATs much greater than 3xULN, some cases also show elevation of serum TBL to ≥2xULN, without initial findings of cholestasis (manifested by a substantial increase in serum alkaline phosphatase activity (ALP)).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C, preexisting or acute liver disease, or another drug capable of causing the observed injury.

Finding one "Hy's Law" case in clinical trials is ominous; finding two is highly predictive of a potential for severe DILI. Clinical trials of the beta blocker dilevalol (enantiomer of labetalol, a diastereoisomeric mixture), showed two such cases in about 1,000 exposures. The drug was not approved in the United States, and examination of a postmarketing study in Portugal revealed fatal liver injury. Clinical trials of tasosartan, an angiotensin II blocking agent, showed a single "Hy's Law" case. The manufacturer was asked to do a large-scale safety study before the drug could be approved.

As a rule of thumb, based on Zimmerman's original estimate of 10 to 50 percent mortality associated with hepatocellular injury sufficient to impair the liver bilirubin excretory function, one can estimate that severe DILI may occur at a rate of about one-tenth the rates of so-called "Hy's Law" cases (Temple 2001). This observation was recently confirmed in large studies of

DILI in Spain (Andrade et al. 2005) and in Sweden (Björnsson and Olsson 2005) in which approximately 10 percent of patients with hyperbilirubinemia or jaundice died or needed liver transplants.

Recent examples of some drugs causing idiosyncratic hepatotoxicity (bromfenac, troglitazone, ximelagatran) further illustrate the predictive value of "Hy's Law," where findings during clinical trials were noted and severe DILI occurred after marketing. These examples are described in detail in the Appendix.

Past experience, including the three examples, shows that there is a set of laboratory abnormality signals that have the ability to predict a potential for severe DILI with different sensitivity and specificity in the context of a several thousand subject database. Although it is not yet possible to provide precise specificity and sensitivity estimates for the various signals, it is nonetheless possible to offer some reasonable guidance on use of these major indicators of a potential for severe DILI:

1. *An excess of AT elevations to >3xULN compared to a control group.*
   AT elevations to >3xULN are relatively common and may be seen in all groups, but an excess of these elevations compared to a control group is nearly always seen for drugs that ultimately prove severely hepatotoxic at relatively high rates (1/10,000). Therefore, the sensitivity of an excess of >3xULN AT elevations as a predictor of a potential for severe DILI is high. But many drugs show this signal without conferring a risk of severe injury (tacrine, statins, aspirin, heparin, and others), indicating low specificity for AT elevations alone.

2. *Marked elevations of AT to 5x-, 10x-, or 20xULN in smaller numbers of subjects and not seen in control groups (or seen less frequently).*
   Virtually all severely hepatotoxic drugs show such cases, indicating high sensitivity for predicting severe DILI, but, again, drugs such as tacrine and others also can cause AT elevations to this degree so that specificity of this finding is suboptimal.

3. *A small number of cases of pure hepatocellular injury (no evidence of obstruction, such as elevated ALP in gall bladder or bile duct disease, malignancy) in a setting of an increased rate of AT elevations, with no other explanation (viral hepatitis, alcoholic or autoimmune hepatitis, other hepatotoxic drugs) with even modest bilirubin elevations.*
   The sensitivity of this observation appears high for any given rate of severe DILI if enough people are exposed to the drug. Thus, if the incidence of severe injury is 1/10,000, and the rate of "Hy's Law" cases is 1/1,000, you would need about 3,000 subjects ("Rule of 3") to be reasonably sure of seeing a "Hy's Law" case (Rosner 1995). The specificity of this finding appears very high if at least two cases are seen (dilevalol, bromfenac, troglitazone, ximelagatran). We are not aware of false positive "Hy's Law" findings. The finding of two "Hy's Law" cases, and probably even one, is thus a strong predictor of a significant rate of severe liver injury.

The implications of these three findings may be different in patients with preexisting liver disease or bilirubin metabolism abnormalities, or in patients on drugs for liver disease treatment

or that themselves inhibit bilirubin glucuronidation. These findings may also not be applicable to certain exceptional drugs that appear to behave differently (such as perhexilene, benoxaprofen, fialuridine).

3. CLINICAL EVALUATION OF DRUG-INDUCED LIVER INJURY

3.1 GENERAL CONSIDERATIONS

For most drugs in development that reach phase 3 testing, the chances of encountering severe DILI are low. Had an increased frequency of mild hepatotoxicity (AT elevations) been observed in earlier trials, everyone would be on alert to evaluate liver injury during phase 3 testing. It is critical, however, to determine whether the mild hepatotoxicity reflects a potential for more severe DILI or reflects a capacity for only limited injury. To make this distinction it is essential to detect any cases of more severe injury and to examine such cases closely, observing the course and outcome of the injury and seeking additional information that might identify other causes. The following general recommendations for evaluating and monitoring potential drug-induced hepatotoxicity may not be suitable for all situations and should be modified for special populations, such as people with preexisting liver disease or malignancies, and in light of accumulating data. In addition, clinical trials of cellular and gene therapies and of vaccines pose specific problems related to trial size and design, persistence of vectors, and tissue specificity.

3.1.1 Patient Selection

Patients are sometimes excluded from clinical trials because of baseline liver test abnormalities or a history of liver disease, but there is no well-established reason to do this, except perhaps to avoid confusion between the previous disease and an effect of the test drug. These patients generally should be included in trials because they are likely to be treated with the drug if it is marketed. Preexisting liver disease is not known to make patients more susceptible to DILI (Zimmerman 1978, 1999), but it may be that a diminished "liver reserve" or the ability to recover could make the consequences of injury worse, making it appear that such patients were more susceptible to DILI. If the drug is intended to be prescribed or marketed to such patients after approval, they should be studied during controlled trials.

3.1.2 Detection of DILI

In general, early studies of a drug in study subjects with presumably normal liver function involve obtaining liver tests every 2 to 4 weeks, at least for a few months. It is uncertain whether early symptoms (anorexia, nausea, fatigue, right upper abdominal discomfort, vomiting) precede or follow the first laboratory signs of hepatic injury (rising ALT, AST, or ALP) and the pattern may vary with different drugs and recipients, but in most cases the first evidence of a problem is elevated AT or ALP. In longer trials, if there is no sign of liver injury after a reasonable length of exposure (e.g., 3 months), the monitoring interval is often increased to once every 2 to 3 months. Later trials also may use less frequent liver chemistry monitoring if there is no indication of hepatotoxicity.

If symptoms compatible with DILI precede knowledge of serum abnormalities, liver enzyme measurements should be made immediately, regardless of when the next visit or monitoring interval is scheduled. In some cases, symptoms may be an early sign of injury. Reliance on early symptoms, rather than serum enzyme monitoring, has become the standard for monitoring isoniazid therapy for prophylaxis of tuberculosis and seems to prevent severe liver injury if acted upon promptly (Nolan et al. 1999). Attention to symptoms does not supplant routine periodic assessment of AT, TBL, and ALP in trials of investigational drugs.

3.1.3 Confirmation

In general, an increase of serum AT to >3xULN should be followed by repeat testing within 48 hours of all four of the usual serum measures (ALT, AST, ALP, and TBL) to confirm the abnormalities. There also should be inquiry about symptoms. Serum AT may rise and fall quite rapidly, and waiting a week or two before obtaining confirmation of elevations may lead to a false conclusion that the initially observed abnormality was spurious, or to severe worsening if the initial abnormality was the herald of a severe reaction to follow. The need for prompt repeat testing is especially great if AT is much greater than 3xULN or TBL >2xULN. For outpatient studies, or studies in which subjects are far away from the study site, it may be difficult for the subjects to return. In this case, the subjects should be retested locally, but normal laboratory ranges should be recorded, results should be made available to study investigators immediately, and the data should be included in the case reports. If symptoms persist or repeat testing shows AT ≥3xULN, it is appropriate to initiate close observation to determine whether the abnormalities are improving or worsening.

3.1.4 Close Observation

Close observation is defined as follows:

- Repeating liver tests two or three times weekly
- Obtaining a more detailed history of symptoms and prior or concurrent diseases
- Obtaining a history of the use of concomitant drugs (including nonprescription medications, herbal and dietary supplement preparations), alcohol, recreational drugs, and special diets
- Obtaining a history of exposure to environmental chemical agents
- Obtaining additional tests to evaluate liver function (PT/INR)
- Considering gastroenterology or hepatology consultation

It is critical to initiate close observation immediately upon detection and confirmation of early signals of possible DILI, and not to wait until the next scheduled visit or monitoring interval. A threshold of a 3xULN transaminase level is reasonable, as lesser elevations are common and nonspecific. If additional testing is done, beyond that specified in the study protocol, it is important that the subject's information be added to the case report forms or database.

3.1.5 Decision to Stop Treatment

It has been observed that "dechallenge" (stopping administration of drug) does not always, or even usually, result in immediate improvement in abnormal lab values. Abnormal test values and symptoms may progress for several days or even weeks after discontinuation of the drug that caused the abnormality. For example, rising TBL usually follows serum AT increases by a few days to weeks. The primary goal of close observation is to determine as quickly as possible whether observed abnormal findings are transient and will resolve spontaneously or are progressive. For most DILI, no specific antidotes are available (except N-acetylcysteine for acute acetaminophen overdose if given promptly, and possibly intravenous carnitine for valproic acid hepatotoxicity). Promptly stopping administration of the offending drug usually is the only potentially effective therapy.

A difficult question is when to stop administration of the investigational drug. Because transient rises and falls of ALT or AST are common, and progression to severe DILI or acute liver failure is uncommon, automatic discontinuation of study drug upon finding a 3xULN elevation of ALT or AST may be unnecessary. For most people, the liver appears capable of adapting to injury by foreign chemical substances, which may render a person tolerant to the drug despite continuation of exposure. Stopping a drug at the first hint of mild injury does not permit learning whether adaptation will occur, as it does for drugs such as tacrine that cause liver injury but do not cause severe DILI. On the other hand, continuing drug administration too long can be dangerous once there is marked transaminase elevation or evidence of *functional* impairment appearing after hepatocellular injury, as indicated by rising bilirubin or INR, which represent substantial damage. In general, treatment should be stopped if:

- ALT or AST ≥8xULN
- ALT or AST rises rapidly to >5xULN in less than 4 weeks or persists for more than 2 weeks
- ALT or AST >3xULN **and** (TBL >2xULN **or** INR >1.5xULN)
- ALT or AST >3xULN with the appearance of worsening of fatigue, nausea, vomiting, fever, rash, or eosinophilia

3.1.6 Evaluation of Alternative Causes

One of the critical purposes of close observation is to gather additional clinical information to determine the most likely cause of the observed abnormalities, and specifically whether there is a cause other than the study drug, by gathering additional clinical data. The presence of acute viral hepatitis A, B, and C should always be evaluated by serological markers; alcoholic and autoimmune hepatitis should be assessed by history and serologic testing; biliary tract disorders should be assessed by ultrasound study; and cardiovascular problems of hypotension or right heart failure should be assessed by physical examination and history. Other less common causes also may need to be considered.

The usual onset of hepatocellular DILI is indistinguishable from acute viral hepatitis A or B. Hepatitis C is much less often acute in its onset and tends to be insidious, but it sometimes can resemble acute drug injury. Viral hepatitis D and E are relatively rare in the United States.

Rarely, Epstein-Barr virus and cytomegalovirus cause liver injury, although this is seen more commonly in immuno-suppressed individuals. Acute alcoholic hepatitis usually is recurrent, with a history of binging exposure to alcohol preceding episodes, and it has some characteristic features, such as associated fever, leukocytosis, right upper quadrant pain and tenderness, and AST >ALT, that may help distinguish it from other causes of liver injury. Autoimmune hepatitis may be acute or even fulminant in its onset; it does not always respond immediately to corticosteroids, but may have serological markers of value. Biliary tract disease more often causes cholestatic injury initially and should be investigated with gall bladder and ductal ultrasound study, especially if ALP is increased. Malignant interruption of the biliary tract also should be considered. Cardiovascular disease, especially right heart failure and hypotension, may cause acute centrilobular hypoxic cell necrosis ("ischemic hepatitis"), with spectacular increases of serum AT, but often can be easily ruled out by history and physical examination.

Exclusion of the two "ABCs" (viral hepatitis A, B, or C; alcoholic or autoimmune hepatitis, biliary disorders, and circulatory disorders) as causes of liver injury should be attempted in all cases of suspected DILI, and results recorded. There is a practical limit as to how much testing should be done to exclude less common liver diseases, such as acute Wilson's disease or alpha-1-antitrypsin deficiency.

It is also critical to discover concomitant treatment that might be responsible for injury. Many people take multiple drugs, perhaps less often in controlled clinical trials because of exclusion criteria, but subjects may not report taking disallowed drugs or other agents. The possible exposure to potentially toxic herbal or dietary supplement mixtures of unknown composition, nonprescription medications such as acetaminophen, or to occupational chemical agents may not be volunteered unless subjects are specifically questioned.

3.1.7 Follow-Up to Resolution

All study subjects suspected of possible DILI should be followed until all abnormalities return to normal or to the baseline state. DILI may develop or progress even after the causative drug has been stopped, so it is important that all study subjects be followed for at least 4 weeks after drug discontinuation. Results should be recorded in the case report form and database.

3.1.8 Rechallenge

Whether or not to rechallenge a subject who showed mild DILI is a difficult question. Re-exposure may initiate a sometimes explosive and more severe reaction, as was observed with halothane several decades ago. Some cases of DILI show indicators of immunological reaction such as eosinophilia, rash, fever, or other symptoms or findings, and it is possible that such cases are more prone to recur with re-exposure. On the other hand, most people can adapt to xenobiotic substances such as new drugs, and develop tolerance for them, as has been found for drugs such as isoniazid (for the large majority of those showing hepatocellular injury, but not for all). They can then resume taking the drugs without further adverse consequence, in which case use of rechallenge to verify drug causation would give a false negative result.

Rechallenge of subjects with significant (>5xULN) AT elevations generally should not be attempted, and if attempted should be done with close follow-up. Rechallenge can be considered if the subject has shown important benefit from the drug and other options are not available or if substantial accumulated data do not show potential for severe injury. The subject should be made aware of the potential risk, and consent to the rechallenge.

3.1.9 Research Opportunities

It is not known why only a few people show severe DILI in response to a hepatotoxic drug while others show nothing or seem to adapt. The current thinking is that there may be a genetic basis for such differences, but acquired factors may be equally important. The period of close observation provides a major opportunity to gather and store serial samples of blood and urine, to investigate characteristics of subjects who show evidence of DILI, and to see how they differ from other people who do not show such effects despite being similar in age, sex, and exposure to the drug. These serial samples can be studied by genomic, proteomic, and metabolomic methods to determine how subjects differ, and to seek biomarkers that identify the susceptible persons.

As part of the Critical Path Initiative, the FDA is working with industry, academia, and other experts to broaden our understanding of the biochemical and genetic bases of drug-induced liver injury. In June 2006, the FDA co-sponsored a scientific workshop to determine the feasibility of developing an in-silico model for DILI from which other predictive experimental models can be derived to characterize potential hepatotoxicity. The long-term goal is to develop a model, or models, that can help researchers identify criteria for determining when early clinical intervention is appropriate. It is also hoped that predictive bioassays and biomarkers can be identified that will help determine which patients most likely will suffer liver toxicity from specific compounds.

This urgently needed research is not a regulatory requirement, but is an important opportunity. The goal is to be able to identify persons who should never be exposed to a given drug because they are idiosyncratically hypersusceptible to, or unable to recover from, DILI caused by it. If tests that screen for people susceptible to severe DILI can be developed, the drug can remain available to people who are not susceptible to severe DILI, instead of having it withdrawn from the market allowing no one to benefit from it.

3.2 CASE REPORT FORMS

In addition to collecting information on laboratory abnormalities, clinical symptoms, and the potential cause of any hepatic illness, case report forms should include the following information for cases in which liver injury is found (including control subjects with such injury):

- Time/date from start of drug administration to start of illness
- Time/date of cessation of drug, or interruption of drug administration
- Space for recording free text to describe the course of illness, including abnormalities of transaminases, ALP, and TBL

- Risk factors, especially alcohol use history
- Use of all concomitant drugs (dose, start and stop dates, whether drug is known to be hepatotoxic, rechallenge/dechallenge information)
- Evaluation of nondrug causes: recent hepatitis A, B, C serology, evidence for biliary obstruction, acute alcoholic hepatitis (AST >2xALT), recent history of severe hypotension or congestive heart failure, underlying other viral disease
- Rechallenge/dechallenge information with suspect drug, with details of time and dose
- All supplemental information, including tests in local laboratories, unscheduled tests and physical exam reports, consultation reports, narrative information, and special studies

3.3 INTERPRETATION OF SIGNALS OF DILI OR ACUTE LIVER FAILURE

3.3.1 Frequency and Magnitude of Liver AT Abnormalities

The presence of even a single case of severe liver failure resulting from treatment in the premarketing clinical trials database is an obvious indicator of a high level of hepatotoxic risk. More commonly, however, there will be no identifiable cases of severe liver injury, but rather varying degrees of serum AT abnormalities that need to be interpreted. As previously noted, slight abnormalities of this kind (to <3xULN) are common in untreated and placebo-treated subjects and are not informative about the potential for the development of severe DILI.

It has therefore become standard practice to look at greater deviations, such as AT values ≥3x-, 5x-, or 10xULN. Because these abnormalities can occur in placebo-treated groups, it is important to compare their rate in drug-exposed subject groups relative to control groups (i.e., placebo or products that do not cause elevation of transaminases). An excess of AT abnormalities >3xULN is a signal of a potential for severe DILI, but, even though it has high sensitivity, it is not specific. Comparison of rates of AT elevations during drug treatment to a control group is probably less critical for abnormalities of greater magnitude (e.g., 10xULN) as such elevations are rarely seen spontaneously. These greater AT elevations can therefore be examined in the whole clinical trials database, not just in the controlled trials. It should be appreciated that serum AT activity is a relatively volatile measurement, often rising and falling within days. It cannot be concluded from one measurement that a peak value has been seen, so that detection of an abnormal rise is a call for serial measures to determine which way the abnormality is moving, whether increasing or decreasing.

A number of factors may confound interpretation of AT abnormalities seen in NDA or BLA databases. Although the more extreme AT elevations may be better predictors of toxicity than smaller elevations, it is possible that close monitoring could affect the magnitude of abnormalities seen if it leads to earlier cessation of drug treatment that prevents the greater abnormalities from appearing. In addition, the contribution of drug treatment to an exacerbation of preexisting liver disease may be difficult to determine. Finally, normalization of abnormalities on continued treatment is not proof that the abnormality was not drug-caused, but may result from liver adaptation to the drug.

3.3.2 Combined Elevations of Aminotransferases and Bilirubin

When AT abnormalities indicating hepatocellular injury are accompanied by evidence of impaired hepatic function (bilirubin elevation ≥2xULN), in the absence of evidence for biliary obstruction (i.e., significant elevation of ALP) or some other explanation of the injury (viral hepatitis, alcohol hepatitis), the combined finding (i.e., "Hy's Law" cases) represents a signal of a potential for severe DILI. Experience has indicated that the occurrence of even two cases of this combination is ominous, indicating a likelihood that the drug will cause severe liver injury.

The absence of "Hy's Law" cases in an NDA or BLA database may allow an estimate of an upper limit of the rate for severe DILI, using the "Rule of 3" derived from simple binomial calculation. There will be at least a 95 percent chance of seeing one or more cases of DILI in 3n study subjects if its true incidence is one in **n** subjects, and the group is well observed. Thus, if no cases of AT and bilirubin elevations are seen in 3,000 well-observed subjects, it can be concluded with 95 percent confidence that the true rate of such occurrences is not more than 1 per 1,000. This calculation would then place a probable upper limit on the rate of expected severe liver injury at approximately 1 per 10,000 exposed patients, assuming that the rate of severe injury when AT and TBL are both elevated is about 10 percent.

3.4 ANALYSIS OF SIGNALS OF DILI

Based on our experience, we recommend that the following analyses related to liver injury potential be carried out and included in an NDA or BLA, or included in an IND when DILI is suspected and being evaluated.

3.4.1 Assessment of Drug Metabolism

The metabolism of a drug can have serious consequences for the safety profile of the drug. A drug may be metabolized to a hepatotoxic metabolite (e.g., acetaminophen, halothane, and isoniazid). Most hepatotoxic drugs have been oxidatively metabolized by the CYP450 system.

Several in vitro methods are available to detect and quantify binding for a drug or its metabolites to liver proteins, including radiochemical and immunological methods.

3.4.2 Assessment of Liver-Related Adverse Events in Controlled Trials

Analyses of incidence rates of abnormal AT, bilirubin, and ALP levels seen in subjects in controlled trials with at least one dose of drug exposure should be provided, generally for pooled data, although study-to-study differences may be of interest. Rates can be given as the number of events/number of subjects exposed, or as the number of events/subject-years of exposure, preferably both. For drugs that appear to require a minimum duration of exposure before DILI occurs, it is useful to give the rates for subjects who have had the minimum duration of exposure (e.g., rate in subjects with at least 1-month exposure). Rates for pooled data should include (but are not limited to):

- 3x-, 5x-, 10x-, and 20xULN elevations of AST, ALT, and either ALT or AST
- Any elevations of bilirubin; elevated bilirubin to >1.5xULN, and to ≥2xULN
- Any elevations of ALP ≥1.5xULN
- Elevation of AT (≥3xULN) accompanied by elevated bilirubin (>1.5xULN, ≥2xULN)
- Possibly liver-related deaths and liver-related treatment discontinuations. These should be described and time-to-event analyses should be performed on these cases. Follow-up status should also be provided. There should be a description of any rechallenge data.

All rates should be calculated separately for drug, placebo, and active-control groups. Normal ranges for all tests should be provided. Time-to-event analyses for elevated rates of significant individual events (e.g., elevated AT, bilirubin) should be provided. The contribution of sex, age, risk factors, and drug dose or regimen to the abnormalities seen should be explored.

3.4.3 Assessment of Liver-Related Adverse Events in the Entire Clinical Trials Database

Analysis of rates of abnormal AT, bilirubin, and ALP levels for the total clinical trials database, including subjects with exposure of at least one dose of study drug in phase 1 or phase 2 trials, or in uncontrolled, open label, extension trials should be provided. We recommend the same evaluation as for the controlled trials database discussed in section 3.4.2. Time-to-event analyses, mortality rates, study withdrawals, and similar data should be provided for significant abnormalities. The contribution of sex, age, and drug dose or regimen to the abnormalities seen should be explored.

3.4.4 Assessment of "Hy's Law" Cases in the Clinical Trials Database

NDA and BLA submissions should include a listing of "Hy's Law" cases identified by treatment group (e.g., subjects with any elevated AT of ≥3xULN, without more than a slight ALP elevation, and associated with an increase in bilirubin ≥2xULN). A narrative summary for each "Hy's Law" case should be provided. Narrative summaries should not only provide, in text format, the data that are already presented in the case report tabulation, but also should provide a complete synthesis of all available clinical data and an informed discussion of the case, allowing for a better understanding of what the subject experienced. For a narrative summary to be useful, it should contain the following information:

- Subject age and sex
- Discussion of signs and symptoms related to hepatotoxicity
- An assessment of the relationship of exposure duration to the development of the adverse event
- Pertinent medical history
- Concomitant medications with start dates relative to hepatotoxicity
- Pertinent physical exam findings
- Pertinent test results (e.g., lab data, biopsy data) shown in tables and/or figures, if possible
- The time course of transaminase and bilirubin elevations, preferably using graphical displays

- A summary of all available clinical information including, if known:
  - Prior or current history of ethanol use
  - Evidence for pre- or co-existing viral hepatitis, or other forms of liver disease
  - Symptoms and clinical course including follow-up to resolution
  - Special studies, radiologic examinations, liver biopsy results
  - Presence or absence of possible confounders, including concomitant illness, use of concomitant medications that are known hepatotoxins, such as acetaminophen
- Discussion of hepatotoxicity as supported by available clinical data and overall assessment of treating physician, consultants, and applicants
- Treatment provided
- Rechallenge and dechallenge results, if done
- Outcomes and follow-up information
- Hospital discharge summaries, pathology, and autopsy reports

The availability of liver biopsy, explant, or autopsy slides for pathology review by review staff or external expert consultants has been helpful in the FDA's assessment of such cases. Reports of external consultant opinions solicited by the applicant should be provided to the FDA.

Complete narrative summaries that include the components listed above also should be provided for all subjects who died of hepatic illness, or who discontinued study drugs for reasons of hepatotoxicity, including subjects with abnormalities consistent with protocol-specific stopping rules.

3.4.5 Overall Assessment of a Drug's Potential to Cause Hepatotoxicity

The overall assessment should characterize a drug's potential for DILI and should consider at least the following questions:

- Was liver monitoring sufficient to characterize DILI risk?
- Were there any cases of probably drug-induced severe DILI?
- Were there signals of a potential for DILI (AT elevations, "Hy's Law" cases) and how were these assessed?
- What doses and durations of exposure were associated with hepatotoxicity signals?
- What approximate incidence of mild and severe DILI could be expected postmarketing?
- Is there sufficient information from the trials to inform an overall risk-benefit assessment?
- Was there sufficient drug exposure (number of study subjects and duration of treatment of each study subject) and adequate liver test monitoring to reliably set an upper boundary for risk of severe DILI after marketing?
- What rate of severe injury (assuming "Hy's Law" cases occur at about 10 times the rate of severe injury) has been suggested or has been ruled out (e.g., no "Hy's Law" cases in 3,000 subjects implies a rate of such cases of <1/1,000 and thus a rate of severe DILI of <1/10,000). This consideration should reflect the presence or absence of other signals, such as marked elevation of AT.

- Will some form of monitoring, by symptoms or serum testing, be needed? Usually, this would be considered only if there was evidence of severe liver injury or the potential for it. If that is the case, the success of monitoring in the NDA database should be discussed.

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5. APPENDIX — ILLUSTRATIVE EXAMPLES

5.1 DURACT (BROMFENAC)

Bromfenac was a nonsteroidal anti-inflammatory drug (NSAID) studied for both short-term analgesia and long-term arthritis treatment. There was little evidence of hepatotoxicity in the short-term analgesic trials, but during clinical trials in arthritis, ALT elevations >3xULN were seen in 2.8 percent of subjects on bromfenac, compared to none in placebo group (Goldkind and Laine 2006). There were two cases in which there was elevated TBL as well as AT elevation in the clinical trial data submitted for review in the NDA. Concerns about possible liver toxicity led to approval of bromfenac for short-term use only and not for osteoarthritis or rheumatoid arthritis. As an NSAID, however, it was prescribed long-term off-label in arthritic patients, and severe hepatotoxicity emerged. Within 6 months of approval, reports of severe hepatic failure, including two cases requiring liver transplant, were received. All severe cases involved the use of bromfenac for more than 10 days, the maximum duration of treatment recommended in the labeling.

In response, the FDA and the manufacturer strengthened the warnings in the package insert with a boxed warning, and issued a Dear Health Care Professional letter. Despite these efforts, the manufacturer and the FDA continued to receive reports of severe injuries, including reports of death or need for liver transplantation (Moses et al. 1999; Hunter et al. 1999; Rabkin et al. 1999; Fontana et al. 1999). Given the availability of other NSAIDs of equal effectiveness and safety, bromfenac was withdrawn from the market in June 1998. The two "Hy's Law" cases in the long-term-exposed population of about 1,000 subjects during drug development predicted an occurrence of severe hepatotoxicity during chronic use at a rate of about 1/5,000 to 10,000 people. Following approval, rates of acute liver failure for bromfenac were estimated to be in the range of 1/10,000 (Goldkind and Laine 2006).

5.2 REZULIN (TROGLITAZONE)

In reviews of the clinical trials of troglitazone conducted prior to approval there were no cases of liver failure among 2,510 subjects exposed to the drug in the NDA database, but 1.9 percent of troglitazone-treated subjects had ALT >3xULN compared to 0.3 percent of placebo-treated subjects, 1.7 percent had ALT >5xULN, and 0.2 percent (5 subjects) had ALT >30xULN (two subjects in the last group also experienced jaundice). The median duration of troglitazone therapy before peak ALT elevation was 121 days. In the Diabetes Prevention Trial at the National Institutes of Health (NIH) performed after approval, 4.3 percent of 585 troglitazone-treated subjects had ALT ≥3xULN, 1.5 percent had ALT >8xULN, and two subjects had ALT >30xULN, compared to 3.6 percent of subjects with ALT ≥3xULN in the placebo group (Knowler et al. 2005). One of the subjects with ALT >30xULN developed liver failure and died, despite receiving a liver transplant. The second subject recovered. These data suggest that the rate of severe liver injury would be about 1 in 3,000 to 10,000.

After marketing, there were numerous reports (Gitlin et al. 1998; Vella et al. 1998; Herrine and Choudary 1999) of acute liver failure associated with troglitazone use, and four letters were sent to practicing physicians between 1997 and 1999, urging monthly monitoring and careful use.

These letters did not significantly affect the monitoring done by physicians, and AT monitoring recommended in the Dear Health Care Professional letters and in the package insert was not regularly performed (Graham et al. 2001). Moreover, an analysis of 94 cases of liver failure reported spontaneously to the FDA showed that the progression from normal hepatic test results to irreversible liver injury occurred in less than a month (the recommended monitoring interval) in 19 patients. The onset of injury began after 3 days to more than 2 years of troglitazone use (Graham et al. 2003a; Graham et al. 2003b). Time from jaundice to hepatic encephalopathy, liver transplantation, or death usually was rapid, averaging 24 days. Troglitazone was withdrawn from the United States market in March 2000, when other agents (rosiglitazone, pioglitazone) with similar efficacy but little or no hepatotoxicity became available.

Apart from constituting another example of the predictive value of evidence of hepatocellular injury accompanied by even two cases of elevated bilirubin, there were other lessons learned from the troglitazone experience: 1) monitoring recommendations, even after several warning letters to all practicing physicians, may not be well followed; and 2) some cases of severe hepatotoxicity occur rapidly, within less than a reasonable and practical recommended interval for monitoring, indicating that monitoring would provide at best only partial protection, even if recommendations were followed. In addition, many companies began to search for toxigenomic answers to determining individual susceptibility to DILI, and a national network was funded by NIH in 2003 to study the problem (Watkins 2005).

5.3 EXANTA (XIMELAGATRAN)

EXANTA (ximelagatran), an oral anticoagulant (antithrombin), was not marketed in the United States because of hepatotoxicity and other concerns discovered during clinical trials. Issues related to potential liver toxicity of ximelagatran were presented and discussed at an FDA advisory committee meeting in September 2004 (He 2004). During short-term clinical trials of the drug for prevention of thromboembolic complications after joint replacement surgical procedures, there was an increased rate of transaminase elevations in the ximelagatran group compared to the enoxaparin-warfarin group, but no serious hepatotoxicity was seen. But in longer-term (>35 days) trials in patients with chronic atrial fibrillation to prevent embolic or thrombotic strokes, an increase in ALT >3xULN occurred in 7.6 percent of 6,948 patients compared to 1.1 percent of patients receiving warfarin treatment; and 1.5 percent of ximelagatran-treated patients had ALT >10xULN.

Increases in AT typically occurred 1 to 6 months after the initiation of ximelagatran administration with peak levels within 2 to 3 months post-randomization. Among the 531 ximelagatran patients with ALT >3xULN, 39 percent completed the study on treatment, while 61 percent discontinued the drug. Almost all patients with ALT >3xULN returned to <2xULN whether the drug was stopped or not, although the return to normal was faster if ximelagatran was stopped. Of 18 patients who resumed drug after ALT returned to normal, only two had elevations recur. Concomitant elevations of ALT >3xULN and bilirubin >2xULN were observed in 37 of about 7,000 patients, at least 13 of whom had no alternative explanation for the concomitant ALT and bilirubin elevation. Nine of the 37 patients died, but the deaths were not clearly hepatotoxicity-related in most cases. Only one autopsy was done and it showed a small, friable and diffusely mottled liver suggestive of severe diffuse hepatic necrosis, but liver failure

from ximelagatran might have contributed to some of the other deaths (He 2004; Lewis 2006; Kaplowitz 2006; Senior 2006; Temple 2006). Because severe hepatotoxicity was observed during foreign marketing, EXANTA was withdrawn in February 2006 from the 22 countries in which it had been approved, and further development in the United States was abandoned.

Again, short-term tolerance of ximelagatran, with resolution of even substantial elevations of ALT in most cases did not predict long-term safety. The relatively high rate of "Hy's Law" cases, about 0.2 percent or 1/500 (13 cases among 7,000 exposed patients), predicted the occurrence of severe hepatotoxicity, at a rate of about 1/5,000 (10 percent of the rate of "Hy's Law" cases). In fact, at least one death occurred among the 7,000 exposed patients subsequent liver toxicity, further supporting such an estimate.