

**Improving Early Drug Development by Accelerating the Advancement
of Tools to Assess and Predict Renal Safety**

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**Draft White Paper for the Institute of Medicine Meeting on October 24,
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Safety.**

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Summary

In the current state, findings of treatment related histologic injury to the kidney in animal toxicology studies rank among the top reasons for attrition of promising therapeutic agents from the drug development pipeline. The lack of sensitive endpoints that could be useful for monitoring kidney disease in current medical practice, furthermore, hampers our present abilities to recognize early when therapeutic interventions of certain complex diseases may, over longer times, beneficially arrest the deterioration of kidney function or even lead to the reversal of disease processes and significant improvements in the health of the kidney. Research progress to identify, evaluate, and qualify improved biomarkers of kidney injury and function would therefore be expected to positively impact both of these areas in drug development. **A vision for the future of drug development to eliminate drug safety issues involving the kidney and to improve monitoring of kidney health** is presented. These descriptions of the

current situation and the vision for the future are followed by: 1) a description of 4 major areas for targeted accomplishments, **what is currently being accomplished**, where, and by whom, and, 2) for each area, a set of bullets which proposes **the major questions, and major hurdles, obstacles, or information gaps that need to be addressed** to answer those questions and accelerate progress toward meeting the vision. The goal of this meeting is for the assembled group of experts to: a) critically assess the current state, the vision, the areas of targeted accomplishment, and the listed key questions, obstacles, hurdles and gaps, b) identify and prioritize additional questions, obstacles and gaps that may need to be addressed, and c) most importantly, to brainstorm and recommend both strategic and tactical steps that must be taken on the path forward to address these obstacles and gaps. The 4 targeted areas for discussion include: 1) the adoption of in vitro toxicology counter-screen model systems that could be applied early during lead optimization to select out those agents with the highest kidney toxicity potential and also to assess species specificity questions relating to kidney toxicity potential, 2) coordinating qualification initiatives for new translational kidney safety biomarkers for bridging between animal toxicology testing and early clinical investigations, 3) addressing the "Flawed Gold Standard" data analysis conundrum which plagues the fair assessment of new biomarkers that can outperform current standard of practice endpoints, and 4) structuring incentives and expanding the freedom to deploy investigations of new safety biomarkers in all phases of regulated product development.

The Current State:

A) Drug toxicities seen in animal studies account for greater than 30% of the compounds that are discontinued from drug development across PhRMA and pathology based safety findings from animal studies may now be the number one reason for overall drug candidate attrition (Kola ref). Renal injury findings (primarily tubule cell injury), often species specific, are noted to be the second leading cause of those animal toxicity based attritions in some companies. Since the liver and kidney are the major sites of drug metabolism and elimination, it may not be surprising that metabolic disposition processes, high tissue exposures, and accumulations of chemicals might be expected to produce in these organs the highest incidence of dose dependent toxicologic findings across animal toxicology studies that are designed to define target organ toxicities. Promising drug development candidates are not infrequently dropped from early drug development when renal injury is seen in an animal toxicology study test species by microscopic histopathologic examination but no change in current routine parameters (e.g., BUN, serum creatinine) for monitoring safety is noted. Current widely used traditional biomarkers for monitoring such *histologic damage* findings are really biomarkers of overall *renal function* and because of the known large amount of renal reserve that exists, would not be expected to be sensitive indicators of such structural injury caused by drug candidates. Without more sensitive biomarkers of acute changes in renal function or structural damage, these drug development scenarios represent non-monitorable toxicities and the development of such compounds is often suspended, even when human relevance is often highly questionable, but difficult to prove. Knowing that

adverse findings from any one species in animal toxicology studies are imperfect and in fact, approximately 40 - 60% predictive of overall toxicities seen in human trials (Olson H, et al), promising compounds are likely being terminated when adverse kidney effects in humans are unlikely. Because animal studies are poorly predictive, shifting the drug development paradigm to include study designs such as the eIND clinical study with earlier reliance on human test data for critical drug development decision making makes good sense as long as patient safety is ensured at relevant therapeutic doses and exposures. Such circumstances described above result in much wasted time in identifying the best new therapeutic targets to invest in for advancing the best of promising new therapeutic agents into patients with significant medical needs, and in many wasted resources spent on synthesizing and testing agents that are doomed for eventual discontinuation. Those early development chemistry and testing resources could be redirected to other promising druggable targets and compound development projects if tools were available to allow continued clinical development where the human test data would be definitive and those agents safe for clinical development could be advanced, minimally, toward the answers needed from definitive proof-of-principle, or proof-of-concept clinical study endpoints. So, in the current state we are presently deficient in our abilities to: a) screen compounds to predict, and avoid choosing those agents that would yield human relevant kidney toxicities, or to demonstrate that certain instances of kidney toxicities are species specific and human irrelevant, and, b) deploy bridging biomarkers in animal toxicology as well as early clinical studies that can confirm and ensure human renal safety while proving that certain instances of kidney toxicities seen in animal testing are species specific and human irrelevant.

B) Many companies are working to develop new drugs to therapeutic targets that are expected to benefit patients with diseases such as diabetes, hypertension, obesity, heart failure, hyperlipidemias, transplant rejection etc., where deterioration in kidney function is a known co-morbidity (see Table 1). Since the current "gold standard" biomarkers of kidney function and structure are known to be insensitive, patients on trials being given efficacious drugs to combat these diseases may experience some amelioration of pathogenic processes underlying the slow insidious progression of kidney degeneration seen with these diseases. It is presently very challenging to establish early definitive evidence based on objective measures of improvement in kidney health following successful intervention of other mechanism based causes of these multi-organ diseases. The association of any new biomarkers with their ability to reflect any improvement in the kidney's state of health in those diseases remains unqualified for regulatory decision-making purposes even though for some diseases, certain biomarkers are known to be associated with disease progression and prognosis, for example albuminuria and diabetes (ref). Apart from being able to use such data for regulatory decision making, even for internal company decision making to provide confidence for continued drug trials, data that provide an early understanding of such potential links is presently lacking, would be extremely valuable, and are highly desired.

Table 1. Examples where Kidney Damage is known to Follow Progression of the Primary Disease

Primary Disease	Secondary Kidney Disease
Diabetes	Diabetic nephropathy
Obesity	Focal segmental glomerulosclerosis
Hypertension	Nephrosclerosis
Acute heart failure	Acute renal failure
Kidney transplant rejection	Chronic allograft nephropathy

Table 2. Summary of Present State Deficiencies regarding Kidney Safety Issues in Early Drug Development

1. No validated rapid thruput model systems for screening compounds for human relevant kidney toxicities, or for demonstrating that kidney tox seen in test species are human irrelevant.
2. Shortage of qualified translational kidney safety biomarkers that outperform sCr and BUN to dispel concerns that a renal histologic observation in animals is human relevant.
3. New biomarkers are not qualified for assessing improvements in kidney health in those diseases where kidney diseases are known co-morbidities.

Vision of the Future State:

A) Metabolically competent in vitro test systems for rodents, non-rodents *and humans* are available for compound screening to both predict human risk early for the most common kidney toxicity (tubule cell injury) and for assessing whether species specific or human relevant.

B) Small numbers of rodents and nonrodents can be assessed easily in a short term toxicology test using a combination of tissue and accessible biomarkers (e.g., genomic, metabolomic, protein, imaging biomarkers) for predicting a dose dependent acute drug induced kidney injury liability potential defined as dysfunction, anatomic alteration or structural damage seen at later time points.

C) For those test agents where species specific and human irrelevant kidney injury liabilities are suspected, accessible qualified biomarkers are available to establish monitorability early, to more precisely diagnose specific anatomic region involvement and severity, to signal the need for early intervention, and allow complete reversibility. Sponsors are empowered by regulatory authorities to demonstrate the lack of relevant dose dependent human kidney injury potential using a multiplex of new translational qualified bridging safety biomarkers in a definitive early clinical trial that provides coverage of multiple diverse facets of histologic alterations impacting kidney structure and function.

D) For those therapeutic areas where kidney disease is a known comorbidity, new qualified biomarkers are available to assess efficacy and deploy: a) in Phase 2 trials, and b) in Phase 3 marketing approval trials using early secondary signals of potential benefits of a test agent to monitor more acute changes that could inform more chronic kidney disease progression, arrest, or improvement. For the purposes of this workshop which is focused more specifically on biomarkers of drug induced kidney toxicities, this fourth future vision area, while very important, will not be dealt with as extensively in our planning discussions.

Table 3. Summary of the Vision for Future Drug Development where Kidney Health Concerns can be Better Addressed

1. Metabolically competent in vitro screening systems predictive of both human relevant and species specific kidney toxicities
2. Resource sparing short study designs incorporating accessible and tissue biomarkers for both rodent and non-rodent toxicology studies that predict kidney toxicity
3. Translational qualified biomarkers are available for monitoring kidney safety in longer animal toxicology studies as well as early human clinical trials
4. Qualified kidney safety biomarkers can also be used to demonstrate benefits of test agents directed against diseases with kidney co-morbidities

Current Efforts:

1) Coordinating Qualification Initiatives of New Translational Kidney Safety

Biomarkers. Numerous promising translational biomarkers of acute renal damage have been observed and noted in published studies in both humans and animals (Table 2). A number of groups are collaborating to advance our collective understanding of these biomarkers for several specific uses (Table 3), some involving early drug development. It is becoming clear that these new biomarkers are each contributing some unique and specific information that will likely contribute to an overall assessment of the biologic state of kidney function, structural perturbation, and the healing response. A systematically collected finite data set is expected to allow certain of these candidate biomarkers of renal damage to gain broad acceptance and a favorable qualification decision, recognized both by drug development and regulatory scientists for monitoring and ensuring renal safety in regulated early clinical human trials when cause for concern has been raised earlier in drug development.

Key Questions:

- 1) Which data are critical and pivotal to getting us to the point of clinical qualification?
- 2) How can we best utilize existing published data and experimental data in both animals and humans?
- 3) How can we best collaborate to agree, and most efficiently generate any needed new data sets and set reasonable expectations and standards of critical data review?

Major hurdles, obstacles and gaps to be addressed:

- Optimal funding solutions and optimizing public-private partnership structures to maximize communication and collaboration through shared resources – endpoints, assays, samples, data.
- Improving the linkage across groups with common interest, motivation, critical samples, assays, funding
- Coalescing, structuring, and positioning a critical expert review of existent published data to minimize gaps for new data to fill – the relative roles expressed for observational studies / retrospective studies/ prospectively designed outcome based studies
- Running pivotal prospective *outcome based* clinical trials as needed to assess relative performance of new biomarkers in real time
- Maximizing use of fresh samples as well as archived/frozen samples from pivotal studies to minimize resource drain and maximize the knowledge gained
- The pragmatic need for biomarker threshold setting – statistical thresholds based on variance/ medical thresholds based on experiences within assorted study populations; use of intersubject and intrasubject variability data of normal controls
- Statistical multiplicity testing correction concerns vs pragmatic resource constraint issues

- Bringing clarity of understanding to biological interpretations that can be gleaned from studies with new biomarkers of: 1) kidney anatomical region involvement and kidney cell types perturbed, 2) histopathologic processes involved, 3) functions perturbed, 4) time course limitations
- Issues surrounding commercial development of biomarker assay panels for research and development human and animal study use (technical and fiscal issues surrounding the identification, validation, commercial development and acceptance of multi-marker panels) – how to choose which ones to develop for maximal commercial accessibility?
- Focusing all of the output from various and diverse work streams into the domain of key regulatory decision and policy makers while maintaining the full attention and awareness of drug developers throughout the process. How do we ensure a transparent "coronation process" for qualifying new safety biomarkers
- How can we arbitrate apparent "irreconcilable differences between regulatory authorities and drug development sponsors on fundamental core aspects of biomarker qualification procedures?

Table 2. Promising Accessible Biomarkers of Acute Renal Damage or Dysfunction to Complement BUN and Serum Creatinine

<u>Biomarker</u>	<u>Proposed Structural/Functional Interpretations</u>
Albumin	Tubular epithelium functional disturbance biomarker. Small quantities are filtered and efficiently reabsorbed by tubular epithelium.
α -GST	Tubular epithelium cell membrane disruption and cytosol leakage
KIM-1	Tubular epithelium dedifferentiation and regenerative repair response
NGAL	Distal tubule rescue signal to bind deleterious substances, limit damage, promote survival and proliferation
TFF3	Decrease in concentration removes cellular maturation signaling, allowing dedifferentiation
Serum Cystatin C	Functional measure of glomerular filtration and tubular reabsorption.
Urinary Cystatin C	Glomerular damage yields protein overload to lumen to prevent efficient tubular reabsorption process of cystatin C from lumen
β 2Mic	Glomerular damage protein overload to tubular lumen prevents efficient tubular epithelium reabsorption of β 2Mic from lumen
Proteinuria	Glomerular damage functional marker
L-FABP	Anoxia/ ischemia signal in tubular epithelium and potential oxidative damage signal
Clusterin	Tubular epithelium regenerative repair response
NAG	Brush-border enzyme released when damage occurs to tubular epithelium
IL-18	Tubular epithelium protein reflecting initiation of apoptotic cascades
GGT	Tubular epithelium cell membrane disruption
Others???	

Table 3 Consortia and Grant Funded Initiatives to Advance Understanding of the Linkage between Kidney Disease and New Kidney Safety Biomarkers

AKIN – The Acute Kidney Injury Network was formed in 2004 with the overall objective of optimizing outcomes in acute kidney injury by leveraging the resources and perspectives of organizations interested in AKI around the globe. This multidisciplinary collaborative network of members representing about 20 key societies in nephrology and critical care, along with additional experts in adult and pediatric AKI, first convened in 2005 to establish a new definition and staging of AKI. During a follow-up meeting in 2006, the group developed a research agenda to test the utility of the AKIN diagnostic and staging criteria to predict patient outcomes in a variety of clinical settings.

C Path PSTC – The Critical Path Institute Predictive Safety Testing Consortium was formed in 2006 with 16 companies working with academic advisors, FDA, and EMEA to establish and qualify translational bridging biomarkers for monitoring drug induced kidney injury for regulatory decision-making purposes in both animal toxicology studies and early clinical trials

ILSI/HESI Kidney Biomarker Committee – This committee consists of 10 companies collaborating to evaluate promising accessible biomarkers of drug induced kidney toxicities in animals.

InnoMed – European Union funded initiative to foster collaborations between pharmaceutical companies and small and medium sized business enterprises (SME's) that can provide technology solutions, services and study sample measurement data to pharmaceutical groups to advance new safety biomarkers

Intramural NIH Initiatives – NIDDK Workshop on Assessment of Kidney Function and Damage; etc

Extramural NIH Funded Academic Centers and Consortia–Harvard; Cincinnati Children's; TRIBE-AKI (multicenter consortium with Yale as coordinating center), UAB/UCSD O'Brien Core Research Center; etc

2) The "Flawed Gold Standard" Data Analysis Conundrum. Advancing new biomarkers that are needed and expected to improve upon the poor sensitivity of currently available standards presents a major methodological problem for data analysis. When the current "gold standard" for defining kidney damage is a poorly sensitive functional measurement (serum creatinine) then a more sensitive biomarker of acute kidney injury would be expected to change in many instances when serum creatinine will not change. Furthermore under certain circumstances where a test agent may affect renal blood flow and creatinine excretion without injury, serum creatinine may rise under benign conditions without raising critical patient health concerns.

Key Questions:

- 1) How can we resolve in individual patient-by-patient cases as to whether alterations in a new biomarker represents an inappropriate false positive result or the lack of change of serum creatinine represents a false negative "gold standard" alteration?
- 2) How can we resolve in individual patient-by-patient cases as to whether the lack of alteration in a new biomarker represents an inappropriate false negative result or the change of serum creatinine represents a non-injurious pre-renal signal from the "gold standard"?
- 3) What is the appropriate use and interpretation of the Inclusion versus Exclusion ROC analyses?

Major hurdles, obstacles, gaps to be addressed:

- Resolving apparent false positives/false negatives fairly by establishing adjudication committees to judge new biomarker apparent false positives/false negatives versus gold standard false negatives (analogy to IARC)
- The value of lessons learned from animal studies anchored by histopathology
- What additional weight of evidence data need to be collected to help adjudication
- What defines an "outcome" – dialysis, death, transient/sustained sCr increase
- How many different clinical trials are needed to test new biomarkers before gaining general regulatory acceptance (what are the principles here)
- The utility of simultaneous changes in multiple new biomarkers to help adjudicate whether a new biomarker is a false positive or sCr represents a false negative, etc.
- The value, utility, and difficulties inherent in using longitudinal time lag measurements between early alterations in new biomarkers and later sCr elevations

3) Structuring Incentives and the Freedom to Operate to Deploy Investigations of new Safety Biomarkers in Regulatory Product Development Studies. It is in the best interest of both regulatory authorities and drug development scientists to remove ambiguities from drug development and define the appropriate use of new kidney safety biomarkers to enhance both the speed of development of the best therapeutic agents, and monitorability of certain target organs to improve patient safety. The most efficient and theoretically less burdensome approach to discover, to begin to assess the performance of

emerging exploratory biomarkers (on both suspected positive and negative developmental test agents), and to gather control patient data on new biomarkers would be to add such measurements to ongoing animal and clinical trials, yet there is little incentive and many disincentives to adding measurements from unqualified exploratory safety biomarkers or emerging safety biomarkers to such regulated studies (ref book chapter).

Key Questions:

- 1) How can regulatory authorities establish and communicate an official "safe harbor" policy structure to provide such freedom to operate?
- 2) Can regulatory authorities provide some incentives to quickly advance the discovery or evaluation of a declared set of prioritized promising and emerging, but not yet qualified, exploratory safety biomarkers using samples from such regulated studies?
- 3) Can other non-regulatory federal agencies provide general access to samples from certain highly aligned clinical trial sample sets that would allow an early assessment of a prioritized list of new biomarkers?

Major hurdles to be addressed:

- implications for genomics, metabolomics, proteomics, etc., in GLP animal toxicology studies for advancing monitorability and early recognition of numerous target organ toxicities
- disincentives under the current drug development framework
- patient consent issues in clinical trials

- VXDS Guidance? Code of Federal Regulation constraints? Slippery slope?
- Non-disclosure concerns vs early freedom to operate to discover and explore utility of emerging biomarkers that have no bearing on study interpretation or the safe conduct of a clinical trial
- Role for neutral NIH sponsored clinical trials and drug development studies

4) In vitro and other Screening Model Systems: Some in vitro or investigative screening model test systems that minimize compound needs but retain test relevance are needed to definitively rule out human relevant renal toxicities from test compounds being considered for development.

Key Questions:

- 1) Are any such promising test systems close to being established?
- 2) How can their evolution be fostered, and how can they best be fairly and objectively evaluated for predicting animal specific and human relevant kidney toxicities?

Hurdles, Obstacles, Gaps:

- primary cells presently; embryonic stem cell uses in the more distant future – status?
- endpoints/model systems currently available
- status of test agent experience
- consortia/ private companies status?

Participant Expectations

Each participant should help to prioritize where we want to be, prioritize what needs to be done to get there, and critically assess which are the most critical obstacles and data gaps that need to be addressed for each of the 4 categorical "current efforts initiatives underway." For knowledge gaps - prioritize the key scientific questions to address the most critical knowledge gaps – "must know" versus "nice to know". For strategy and tactical obstacles, which are the key component obstacles/gaps and which ones can we realistically address, how do we address them. We will use the input from our discussions to fill out this white paper to provide a set of recommendations that will help to stimulate the path forward for development of improved approaches and tools to reduce kidney toxicity concerns from drug development.

Biomarkers of acute idiosyncratic hepatocellular injury (AIHI) within clinical trials

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BIOMARKERS OF ACUTE IDIOSYNCRATIC HEPATOCELLULAR INJURY (AIHI) WITHIN CLINICAL TRIALS

Major controversies to be addressed

- The extent to which the mechanistic and susceptibility/adaptive factors that characterize severe and idiosyncratic hepatocellular injury in patients, and provide the opportunities for the discovery and validation of predictive biomarkers, are shared among various drugs.
- Whether research efforts in the pre-registration clinical trial space, which excludes most patients susceptible to severe hepatocellular DILI, can lead to the identification of biomarkers that are useful in predicting and managing that rare reaction.

Importance

Hepatotoxicity is the adverse event that most frequently leads to regulatory action on drugs, including failure to approve, postmarketing warnings added to the label, and withdrawal from the market (Temple, 2001). Among research priorities in adverse drug events, hepatotoxicity was ranked first in a 2006 survey of pharmaceutical companies (A. Holden, 2008). The population incidence of drug-induced liver injury is unknown, as most events are inaccurately classified (Aithal 1999). Yet, drug-induced liver injury is the most frequent cause of acute liver failure among those under consideration for liver transplantation in U.S. (Lee 2003). Animal studies (in rodents, dogs and monkeys) detect approximately half of compounds exhibiting hepatotoxicity in man (Olson 2000). In vitro human hepatocyte testing similarly detects 50-60% of drugs that can cause severe liver injury in man, including some not detected on animal testing (Xu 2008). However, no currently available preclinical tests detect potential for serious human hepatotoxicity with combined high sensitivity and specificity.

Recent true case

A major pharmaceutical company submitted an NDA application for treatment of a chronic disease. The FDA agreed with the sponsor's efficacy data. However, it was noted that among ~4,000 treated patients in clinical trials, two developed elevations in both serum alanine aminotransferase and bilirubin. As a prerequisite for approval, the company was told to conduct a new safety study of 10,000 patients treated with drug for one year, and to also include an additional 10,000 subjects receiving comparator treatment for one year. This news will cost the company >\$200M to conduct the trial, ~3 years off patent, and loss of market entry position in class.

Acute hepatocellular injury

The clinical and histological presentation of DILI can take many forms, mimicking most types of liver disease. The major form of liver injury of concern in drug development is acute idiosyncratic hepatocellular injury (AIHI) because of its potential rapidity of development and high morbidity and mortality (Andrade 2005, Bjornsson 2005).

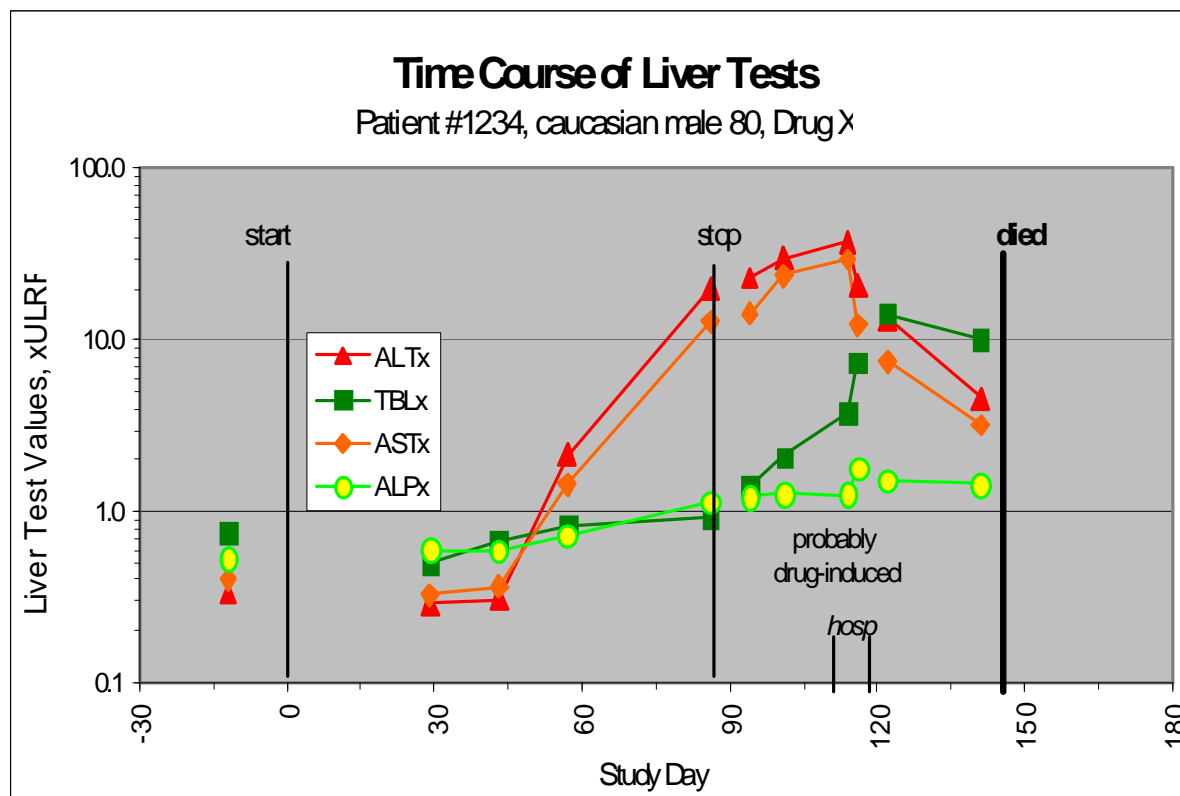
Table 1 lists marketed drugs that have undergone regulatory actions since 1995 for liver safety concerns. All of the drugs listed typically cause AIHI, with the exception of terbenafine (mixed hepatocellular/cholestatic injury), valproate (microvesicular steatosis) and acetaminophen (causes hepatocellular injury but does not have the characteristics of AIHI discussed below). *The IOM session on hepatotoxicity will focus exclusively on AIHI and not other forms of drug induced liver injury.*

Table 1: Regulatory actions on approved due to hepatotoxicity (1995-2008)

<i>Withdrawals</i>	<i>Second Line</i>		<i>Warnings</i>
bromfenac	felbamate	acetaminophen	atomoxetine
trogliptazone	tolcapone	leflunomide	bosentan
pemoline	trovafloxacin	nefazodone	infliximab
		nevirapine	saquinavir
		pyrazinamide/rifampin	
		interferon 1b, 1a	
		terbinafine	telithromycin
		valproic acid	zifirlukast
		(kava, lipokinex)	

A typical presentation of AIHI is shown in figure 1. This patient exhibited normal liver chemistries at baseline and for weeks on treatment, but then developed serious liver injury with loss of overall liver function manifest as a rise in serum bilirubin and ultimately death.

Figure 1



During AIHI, if treatment with the offending agent is not withdrawn promptly, and in some cases even with prompt discontinuation, the progressive loss of hepatocytes leads to liver dysfunction and ultimately death (without liver transplant). The event is frequently termed “idiosyncratic” because the majority of treated patients are able to take the drug safely at the recommended dose range; the affected individuals are different from the majority in ways that make them susceptible or less able to recover from the injury. With most drugs listed in Table 1, fatal AIHI may typically occur in 1:10,000 to 1:100,000 treated patients. It is rare for fatal AIHI to occur in preapproval clinical trials in part because most clinical trials contain insufficient numbers of patients treated for long enough to have a high likelihood of identifying such rare patients. For the purposes of this white paper, we will use the term “*AIHI*” to specifically refer to acute and idiosyncratic hepatocellular injury that can progress to liver failure (ie. The entity depicted in Figure 1).

Types of biomarker sought

For the purposes of the IOM discussion, the primary biomarkers discussed will be those that detect a drug’s potential to cause AIHI in a preapproval clinical trial. Biomarkers to identify individual susceptibility to a drug with established AIHI potential will be discussed to the extent relevant to biomarkers that could be generally applied to clinical trials to detect drugs capable of AIHI. Other types of biomarkers, such as those which may aid in causality assessment, will not be discussed.

Current status of biomarkers for AIHI

Alanine aminotransferase

Serum alanine aminotransferase (ALT) activity is the most frequently used biomarker to detect hepatocellular injury in clinical trials, and is more liver specific than aspartate aminotransferase (AST) (Green 2002). Serum ALT can increase, even markedly (e.g. exceeding 20 times upper limits normal), due to events other than hepatocyte necrosis, including hepatocyte autophagy in anorexia nervosa (Rautou 2008) or hepatic glycogen accumulation in uncontrolled type 1 diabetes (Sayuk 2007, Olsson 1989). Lesser ALT elevations are observed with hepatic steatosis (Browning 2004). Activation of ALT gene transcription can occur in response to PPAR agonists in cell culture (Thulin, 2008). In addition, there is the theoretical possibility that drugs could interfere with ALT degradation or blood clearance. However, no published data support transcriptional or clearance-related ALT increases these events occurring in humans.

Drugs that are recognized to cause AIHI demonstrate increased incidence of ALT elevations > 3 x ULN (relative to placebo or control) in preapproval clinical trials (Temple, 2001). However, ALT elevations have a limited specificity to predict AIHI, as even frequent and fairly high ALT elevations do not reliably predict the potential to cause AIHI in clinical trials, as evidenced by tacrine. About 25% of Alzheimer disease patients receiving tacrine developed ALT elevations >3xULN and 2% exhibited ALT > 20xULN in clinical trials (Watkins 1994). However, tacrine exhibits a very low risk of AIHI. Similarly, although statins exhibit up to a 3% incidence of ALT >3xULN in clinical trials relative to control/placebo, statin use has not associated with an increased risk of acute liver failure (Kaplowitz 2005). Heparins can also cause ALT elevations but have a very low or zero risk of AIHI. Drugs such as tacrine, heparins and statins generally

exhibit transient, self-limited liver injury which resolves with continued treatment in a process termed “adaptation”. Adaptation is not just observed with drugs that have low risk of AIHI, but is also observed in many or most patients who experience ALT elevations while receiving drugs that can cause AIHI, such as troglitazone (Watkins 1998), and isoniazid (Mitchell 1975).

There are likely common pathways that underlie initial injury, and whether the injury progresses or resolves with continued treatment. These pathways include those that determine the intracellular “dose” of hepatotoxic metabolites or bile acids (eg. cellular transporters, Phase 1 and 2 drug metabolism, other concomitant medications), the production of hepatocyte injury (e.g. oxidative stress, mitochondrial impairment, etc.), as well as regenerative or hepatoprotective abilities (including hepatic glutathione redox status, Nrf2 activation of cell defense systems, liver regeneration). all which may be influenced by genetics (Kindmark 2007, Wilke 2007, Daly 2007, Larrey 2002), epigenetics (Murata 2007), demographic (Utrecht 2007, Kaplowitz 2005), infectious/inflammatory (Roth 2003) and environmental influences (Kaplowitz 2005, Larrey 2002). *A popular theory is that progressive liver injury occurs in those individuals that fail to adapt to the identical initial insult.* Data to support this theory are sparse, however. It has been claimed that with drugs capable of causing AIHI, such as isoniazid and ximelagatran, the AIHI events typically occur with a similar latency as the more frequent ALT elevations observed in clinical trials (personal communication D. Larrey, J Utrecht, P Watkins) which is consistent with a mechanistic link between isolated ALT elevations and AIHI events. The temporal relationship between ALT elevations observed in clinical trials and post marketing AIHI events has not been systematically examined.

A logical conclusion from the above discussion is that drugs that cause ALT elevations can be placed along a spectrum with one end being those drugs causing ALT elevations that may reflect liver injury but never cause AIHI. The other end of the spectrum are drugs which cause liver injury that progresses to AIHI with relative high frequency (perhaps one in 10 subjects with ALT elevations who are continued on treatment). While ALT is a sensitive biomarker for liver injury, ALT alone cannot differentiate between drugs at opposite ends of this spectrum.

Drug-induced liver injury with jaundice: the current gold standard biomarker for AIHI potential.

Hy Zimmerman (1968) first noted that a patient who presents with drug-induced hepatocellular injury with jaundice had at least a 10% chance of dying from liver failure (before liver transplantation was available). In hepatocellular injury, a rise in total and direct bilirubin reflects a substantial risk because it indicates a major loss of functioning hepatocytes (when other causes for increased bilirubin are excluded). The approximate 10% mortality or transplant rate for drug-induced hepatocellular jaundice, or “Hy’s Law”, has been confirmed in recent reports from Sweden and Spain (Bjornsson 2005, Andrade 2005).

The Oct 2007 FDA draft guidance for evaluating liver safety in a clinical trial defines “Hy’s Law cases” as subjects in a clinical trial who experience ALT >3xULN and TBL >2xULN and satisfy the following three criteria: 1) the liver injury should be hepatocellular in nature and there should not be a prominent cholestatic component; 2) there should be no more likely alternative cause than DILI, such as acute viral hepatitis A or B, or other acute liver disease; and 3) there

should be evidence that the drug causes more frequent but less severe hepatocellular injury as shown by more frequent ALT elevations greater than 3 xULN in the treated group relative to the group on control treatment. *The agency has placed great confidence in the specificity of Hy's Law Cases as a biomarker for identifying drugs capable of AIHI, reporting that "We are not aware of false positive Hy's Law findings."* (FDA, 2007)

The Hy's Law case is a specific, but imperfect biomarker for drugs capable of causing severe AHI. In an FDA review of 26 new drug applications (of which 13 drugs are known to be "hepatotoxic"), Hy's Law events were seldom observed in the clinical trials, even with compounds later demonstrated to be capable of severe AIHI (Pauls 2004). This is not surprising since the rarity of susceptible individuals and the delayed appearance of the event generally would require very large and prolonged clinical trials for detection. Also, clinical protocols usually mandate frequent monitoring of serum ALT with strict stopping rules based on a serum ALT, especially once a liver safety issue is established for a drug in development. Stopping treatment at a low level of liver injury may allow a patient susceptible to AIHI to recover without demonstrating a rise in serum bilirubin. The only way to determine whether a patient with an ALT elevation will adapt or progress is to continue treatment and observe the patient closely, with frequent monitoring of liver chemistries. The current draft FDA guidance on liver safety (FDA 2007) suggests continued treatment may be considered in subjects with asymptomatic ALT elevations exceeding 3 X ULN. Such practices may place these study subjects at greater health risk than subjects without ALT elevations, raising ethical concerns.

Do better biomarkers than Hy's Law cases exist?

The ideal biomarker would not require placing patients at significant risk in the course of distinguishing between drugs capable of causing AIHI, from drugs like heparin and statins that do not appear to cause AIHI. The ideal biomarker would also be able to make this distinction in a relatively small clinical trial of short duration. The plausibility of such a biomarker rests on the mechanisms that underlie differences between drugs that have the potential to cause severe AHI vs those that cause only reversible ALT elevations. At least two possibilities exist:

- 1) The mechanisms that distinguish AIHI are many, complex and agent-specific to the extent that identifying a manageable number of predictive markers that are applicable to most drugs capable of severe AIHI is impractical or impossible.
- 2) Common mechanisms for AIHI exist and can be translated to a manageable number of validated biomarkers that could be applied to better inform the hepatic safety of candidate drugs pre-registration and in the post marketing setting.

The first scenario suggests the need for drug specific biomarkers for those agents where the risk:benefit balance warrants continued marketing (i.e. agent-specific markers for patients at risk) and when the incidence of liver safety events is high enough to characterize premarketing. The more compelling possibility of "universal" biomarkers, to replace the Hy's Law Case in the prediction of AIHI, relies on the second possibility being correct. What follows are three lines of thought regarding the pathogenesis of severe AHI which can be used to interrogate possible universal biomarkers:

The cumulative injury theory

This theory maintains that drugs capable of AIHI cause a progressive impairment of critical functions of hepatocytes that may start soon after the initiation of treatment, but is not detected by elevation in serum ALT. An example would be progressive mitochondrial injury (e.g. as demonstrated for fialuridine and in cell culture for other drugs such as nefazadone, troglitazone) such that ATP generation is progressively compromised over a period of weeks or months on treatment (McKenzie 1995, Dykens 2008, Xu 2008). When the mitochondrial function deteriorates to a critical level, some hepatocytes start to die, releasing or recruiting injury propagating factors, and/or increasing metabolic demands on neighboring hepatocytes – this nudges neighboring hepatocytes over the tipping point to demise, causing a “domino effect” until liver failure occurs. Some recent data suggest that there may be a small number of critical pathways that compromise hepatocyte function to produce AIHI. In a recent study, human hepatocyte fluorescent imaging was used to examine the effect of 300 hepatotoxins and nonhepatotoxins on mitochondrial damage, oxidative stress, and intracellular glutathione. These *in vitro* studies predicted approximately 60% of drugs capable of AIHI (many of which were not detected in preclinical testing) with a high specificity (0-5% false positive rate)(Xu 2008). Because mitochondrial damage, oxidative stress, and/or depletion of intracellular glutathione may be “downstream” of molecule-specific events, such as reactive metabolite accumulation, a drug capable of severe AIHI could cause characteristic changes in serum proteome or metabolome, or urinary metabolome that would not be present in patients treated with drugs incapable of causing severe AHI.

Immune response theory

Another mechanism proposed to account for the temporal delay in onset and rapidity of liver injury is the production of reactive metabolites resulting in immune activation (Utrecht 2007). Within the hepatocyte, the drug is bioactivated to a reactive metabolite which binds to and modifies hepatocellular proteins. When this modified protein or hapten is then presented by antigen presenting cells to T cells, they transform to cytotoxic T cells and antibody-producing B cells (Park 2005; Kaplowitz 2005). Such drug-induced immune reactions typically occur within the first month of treatment and more rapidly with rechallenge (Kaplowitz 2005), as seen with halothane (Mushin 1971) and may be accompanied by clinical signs of hypersensitivity: fever, rash and eosinophilia. The role of a specific hepatotoxin/metabolite in this immune response can be assessed in some cases by the lymphocyte stimulation test (Sanderson 2006, Kaplowitz 2005). These immune responses may be enhanced by acute inflammation or circulating lipopolysaccharide in rodents (Roth 2003) and it appears that immunoallergic hepatotoxicity is more common in AIDS patients (Kaplowitz 2005). A variety of data suggest that immune mechanisms may underlie AIHI even when there are no clinical signs of hypersensitivity, such as the report of HLA associations with zimelegatran hepatotoxicity (Kindmark, 2007). It is possible that biomarkers of immune activation might be useful in distinguishing benign ALT elevations from those that can portend AIHI. In support for this concept, ALT elevations accompanied by hepatitis symptoms (fatigue, nausea, right upper quadrant pain) appear to be more predictive of AIHI potential than asymptomatic ALT elevations (Nolan, 1999). These

symptoms may be mediated by cytokines or other endogenous proteins, and these proteins may be detectable long before symptoms appear.

Failure of adaptation

If the critical issue in AIHI is failure to adapt to the initial injury, it is possible that there exist biomarkers that could identify patients likely to adapt and conversely to target those progressing to severe liver injury at a very early stage in the injury process.

Sources for candidate biomarkers:

Candidate biomarkers for AIHI are evolving from many lines of investigation, including extensive transcriptomic profiling of rats treated with a variety of hepatotoxic drugs. The Predictive Safety Testing Consortium (PSTC) has been identifying potential liver safety biomarkers, but has not yet focused on detecting AIHI. In the Liver Toxicity Biomarker Study (LTBS), pan-genomic approaches are being used in rats to identify biomarkers capable of distinguishing pairs of drugs that are structurally and pharmacologically similar, but where one compound is capable of AIHI and the other drug is not.

Another path to identifying potential biomarkers for AIHI is the ongoing effort to study patients who have actually experienced AIHI. The Severe Adverse Event Consortium (SAEC) (Holden 2008) has begun whole genome SNP analysis on germ line DNA obtained on patients who have experienced varying degrees of drug induced liver injury, including AIHI. The expanding US based Drug Induced Liver Injury Network (DILIN) (Hoofnagle, 2004) will begin genetic analysis on a similar cohort, and has the advantage of maintaining identity links to the participants such that additional phenotyping studies can be performed. Because subjects are enrolled in these registries only after the diagnosis of DILI injury is made, it is generally not possible to obtain blood or urine early in the course of the liver injury (or prior to the injury).

One research priority will be to generate hypotheses that will be testable in gene banks, and in the DILIN subjects themselves (since the identity link and contact is maintained with all subjects). International drug-induced liver injury registries in U.S., U.K., Japan, Spain, Sweden, and Denmark now contain thousands of expert-adjudicated cases, which can be combined and analyzed for risk factors predicting progression to AIHI. Mining of large postmarketing adverse events databases may also suggest drug/environment susceptibility factors that could lead to testable hypotheses, or be used to provide supportive data for genetic associations observed in these networks. Analysis of blood/urine samples obtained in clinical trials for drugs known to cause AIHI may also be useful in identifying biomarkers, especially when compared to blood/urine samples obtained in clinical trials of drugs that cause ALT elevations but do not have AIHI potential. A large prospective trial in isoniazid treated patients has been proposed for this purpose (Watkins 2008). Studying differences in hepatotoxicity susceptibility across panels of inbred strains of mice and quantitative trait loci mapping may be a promising approach to generating hypotheses that would be testable in relatively small numbers of human subjects.

Finally, the FDA has sponsored a cooperative research and development agreement (CRADA) to develop a computer based model for understanding and predicting drugs capable of causing AHI (http://findarticles.com/p/articles/mi_m0EIN/is_2007_August_6/ai_n19395744). The goal of this effort is to incorporate current mechanistic knowledge, as well as the data and insights

gained from ongoing efforts, such as the SAEC and DILIN analyses. This evolving model might suggest novel biomarkers and/or could provide biological rationale for biomarkers discovered by other means.

Validation of candidate biomarkers:

It is hoped that biomarkers can be found that could distinguish drugs capable of causing AIHI from those that do not in a small clinical trial of short duration. This hypothesis could theoretically be tested by administering examples of both types of drugs to even small groups of closely monitored patients or healthy volunteers and analyzing prospectively collected blood/urine samples. For example, the first pair of drugs studied in the Liver Toxicity Biomarker Study (LTBS) was the COMT inhibitors tolcapone (restricted use due to AHI) and entacapone. Since both drugs are in clinical use, it should be possible to test candidate biomarkers that evolve from the LTBS effort in patients, or possibly healthy volunteers treated with these drugs. Short term healthy volunteer studies of this design may be ethical since the onset of liver injury is typically delayed weeks or months with tolcapone (Olanow, 2007). However, it is possible that the drugs capable of causing AIHI will be distinguishable only once liver injury has begun as signaled by ALT elevations requiring longer term treatment to evoke the phenotype. In this case, blood/urine samples must be obtained from patients with ALT elevations caused by drugs capable of causing AIHI and then compared to blood/urine samples obtained from patients with ALT elevations caused by drugs that do not cause AIHI. True validation of biomarkers would ultimately require large numbers of samples obtained from well-phenotyped individuals including both healthy and diseased populations as well as in populations treated with many different drugs. One path would be to institute protocols for standard data and blood/urine collection once ALT elevations are observed in a clinical trial. One example of a liver safety data management system is eDish (Gelperin 2008). This or similar format could be directly linked to the sample bank, and would allow immediate identification of individual subjects of interest and immediate access to all pertinent clinical and laboratory data for those patients for detailed evaluation. Because the true potential of a drug to cause AIHI may not be evident preapproval, the blood/urine samples and data bank would need to be maintained for some time postmarketing. It would obviously be ideal if scientists now had access to samples and clinical data from the clinical trials of many of the drugs listed in Table 1.

FOCUS OF DISCUSSIONS: CHALLENGES, ASSUMPTIONS AND PROPOSALS FOR DISCOVERY AND VALIDATION OF CLINICAL BIOMARKERS OF AIHI

GOAL:

To find predictive biomarkers for use in clinical development that are better than the Hy's Law Case in predicting AIHI potential for drugs in clinical development.

KEY CHALLENGES

- Low incidence of Hy's Law cases precludes signal detection in most conventional clinical development programs
- Features of the candidate drug (SAR, covalent binding, etc), preclinical safety assessment and *in vitro/in silico* observations, while useful in identifying candidate markers, currently have limited predictive value for AIHI

UNDERLYING ASSUMPTIONS TO BE DISCUSSED:

- Candidate novel biomarkers for predicting and managing AIHI may be best identified by fully characterizing the disorder in patients who fulfill the criteria for a Hy's Law Case, who are very rarely encountered in clinical trials. The scientific community does not currently have access to data or biospecimens from a large proportion of the Hy's Law Cases occurring in clinical practice.
- The second highest potential source for AIHI biomarker discovery may be prospective clinical trials with drugs that are known to have a substantial risk for AIHI, like INH.
- The observation that most or all established AIHI agents have been associated with more benign hepatocellular perturbations in clinical development (that often show a similar temporal pattern) suggests that the two events may be mechanistically related. Fully characterizing clinical trial subjects who experience ALT elevations may provide insights regarding the mechanism of, predisposition and candidate markers for AIHI and/or could provide a relevant negative control population if the tested drug turns out not to have an AIHI risk.
- Because the clinical trial subjects who experience ALT elevations that are mechanistically linked to AIHI may not be readily distinguishable from those with other (more common) hepatocellular perturbations, it will probably not be possible to discern biomarkers that predict AIHI potential from examining these subjects alone. However, candidate markers derived from other research venues, including studies of patients who have experienced AIHI (e.g. DILIN, SAEC), controlled prospective clinical trials on well-characterized AIHI agents (eg INH) and other relevant animal/*in vitro/in silico* models can be used to screen the clinical trial subjects in an attempt to identify, and further characterize potential biomarkers. Once associations are made that confirm the relevance of subjects with isolated ALT elevations for a particular drug, these subjects can ultimately be an invaluable venue for validation of candidate DILI markers.

- Accordingly, the collection and storage of appropriate well-characterized biological specimens from clinical trial patients showing significant ALT elevations (and matched controls treated with drug but not showing ALT elevations) will be critical to making the above prospective and retrospective associations and validating new biomarkers for AIHI

PROPOSAL: CANDIDATE AIHI BIOMARKERS ARE BEST IDENTIFIED AND VALIDATED IN THREE RELEVANT HUMAN POPULATIONS:

1. *Hy's Law Cases*—most relevant population for study, but have limited accessibility for specimen collection or prospective study.
2. *Prospective, controlled clinical trials with established, well-characterized AIHI agent such as INH*—characterize benign, self-limiting hepatocellular effects; compare with patients fulfilling criteria for Hy's Law case either in the same trial (which would have to be very large) or in established registries (eg SAEC, DILIN).
3. *Clinical trial subjects receiving a drug known to cause ALT elevations but not yet known to cause AIHI* – biospecimens obtained may be useful in validating AIHI biomarkers even if the drug is later shown to have a low AIHI risk (negative control).

BASED ON THE DISCUSSIONS OF THE ABOVE, THE FOLLOWING RESEARCH EFFORTS WILL BE PRIORITIZED AND BARRIERS/SOLUTIONS WILL BE IDENTIFIED.

1. Define methods to overcome key barriers to accessing clinical information and biospecimens from Hy's law Cases occurring in clinical practice as the most relevant source of data needed for candidate biomarker identification; identify options/solutions. This would include methods to utilize electronic health records & large datasets (Kaiser, VA, etc.) to identify, characterize and obtain appropriate biospecimens from such patients.
2. Develop and implement protocol(s) for specimen and data collection in a prospective clinical trial(s) of drug(s) known to cause AIHI, like INH. This would include which drugs to target, case report forms, which specimens to collect [serum, urine, whole blood for RNA], timing and frequency of collections, patient population to study, and funding sources).
3. Develop and implement protocols for standardized data and biospecimen collection in Phase 3 clinical trials when an ALT signal is identified. This will include defining criteria to trigger enactment of these protocols, the items mentioned in #2 above, and challenges facing pharmaceutical sponsors and regulators in implementing such a program.
4. Conduct a thorough examination of existing FDA liver safety databases from Phase 3 clinical trials and the AERS database to test the hypothesis that the more frequent but benign ALT elevations occurring in patients receiving an AIHI agent are mechanistically linked to AIHI (*critical assumption the underpins study of clinical trial populations*).

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This would include comparing the clinical characteristics of ALT elevations and post marketing Hy's Law.

5. Prioritize biomarker discovery options using the data and biospecimens obtained from the three populations above, which include but are not limited to established hypothesis-driven associations (immune response, toxic metabolite, cumulative injury, adaptation features, etc); and screening/fishing through application of various molecular profiling technologies, such as candidate gene expression.
6. Identify and prioritize non-clinical research options to generate biomarker hypotheses for testing in the clinical biospecimen banks.

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