

# Next Generation DILI Biomarkers: Prioritization of Biomarkers for Qualification and Best Practices for Biospecimen Collection in Drug Development

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The diagnosis and management of drug-induced liver injury (DILI) remains a challenge in clinical trials in drug development. The qualification of emerging biomarkers capable of predicting DILI soon after the initiation of treatment, differentiating DILI from underlying liver disease, identifying the causal entity, and assigning appropriate treatment options after DILI is diagnosed are needed. Qualification efforts have been hindered by lack of properly stored and consented biospecimens that are linked to clinical data relevant to a specific context of use. Recommendations are made for biospecimen collection procedures, with the focus on clinical trials, and for specific emerging biomarkers to focus qualification efforts.

## FOREWORD

The IQ Drug Induced Liver Injury (IQ DILI) Initiative was launched in June 2016 within the International Consortium for Innovation and Quality in Pharmaceutical Development (also known as the IQ Consortium) to reach consensus and propose best practices on topics related to clinical drug-induced liver injury (DILI) ([www.iqdili.org](http://www.iqdili.org)). The IQ Consortium is a leading science-focused, not-for-profit organization addressing scientific and technical aspects of drug development and is composed of 39 pharmaceutical and biotechnology companies. The IQ DILI Initiative is an affiliate of the IQ Consortium, composed of 17 IQ member companies, focused on establishing best practices for monitoring, diagnosing, managing, and preventing DILI. This publication is focused on the prioritization of DILI biomarkers related to hepatocellular injury in clinical trial development for qualification and best practices for biospecimen collection based on an extensive literature review and the consensus achieved in carefully structured discussions between IQ DILI members and academic and regulatory experts.

## INTRODUCTION

### Current challenges in DILI diagnosis

Occurrence of DILI is a serious safety concern during the development of future medicines and beyond. Diagnosing DILI is challenging because there are multiple clinical patterns and

phenotypes, some of which closely resemble other liver diseases, and the number of over-the-counter medications, use of herbals, and dietary supplements that cause DILI is growing, which makes identity of the causal entity difficult. Furthermore, elevations in aminotransferases may be apparent without any actual presence of a liver injury. Despite these challenges, in many instances a definitive diagnosis of liver injury can be reached and the causal treatment can be stopped. However, it remains difficult to determine whether the patient will progress to severe liver injury or if the injury will resolve over time.

The hepatic injury biomarkers presently available for clinical use are not always adequate to assist a clinician with managing these challenges. With currently available biomarkers for liver injury, tools for causality assessment (like the often used RUCAM (Roussel Uclaf Causality Assessment Method) scale), and even the evaluation by expert opinion,<sup>1</sup> the possibility of false positive assessment forces clinicians to take the most conservative approach in cases of suspected DILI. In addition, mechanistic assessment, the prediction of progression of the injury, and the assignment of which drug or supplement is causing the injury are lacking. While this may help protect patients from harm, it can also lead to discontinuation or suboptimal use of promising therapeutics. When a drug has a proven or suspected DILI concern, specific biomarkers

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Received February 27, 2019; accepted June 12, 2019. doi:10.1002/cpt.1571

that could indicate liver injury earlier than existing biomarkers are lacking.

This burden could be alleviated with the qualification and application of diagnostic and/or predictive biomarkers that were specific to true liver injury caused by medications, herbals, or dietary supplements or that could predict potential severity.

Although the focus of this publication is on hepatocellular injury, there are other important categories of DILI, such as cholestatic liver injury and injury to liver sinusoidal endothelial cells, which was illustrated by the oncology drug gemtuzumab ozogamicin.<sup>2</sup> Other forms of liver injury may be difficult to distinguish from hepatocellular injury. For example, liver sinusoidal endothelial cell injury also leads to ischemic necrosis, resulting in elevated alanine aminotransferase (ALT) levels and histologic evidence of hepatocellular necrosis. The biomarkers described may be applicable to these other categories of DILI and should also be considered in future efforts of liver safety biomarker qualification. Biospecimen archival is imperative regardless of the type of injury.

Successful and efficient qualification of potential DILI biomarkers for use in clinical trials requires three critical conditions:

- Existence of sufficient clinical samples to provide a robust evaluation of the biomarker under consideration
- Existence of a validated, robust assay that will produce accurate, precise, reliable, and reproducible data from these samples
- Selection and prioritization of the most promising biomarkers since qualification efforts could take many years

#### The benefits of collaboration in biomarker qualification

DILI biomarker qualification is challenged by insufficient clinical samples to provide the necessary statistical power for rigorous evaluation, especially for biomarkers capable of predicting idiosyncratic DILI, which has a low incidence rate in geographically defined populations (2.3–14 per 100,000 patients per year).<sup>3,4</sup> There are also difficulties in diagnosis and phenotype assignment. These challenges may be overcome through collaborations between pharmaceutical companies, academicians, and regulators.<sup>5</sup> As an example, 12 out of 14 successful qualifications of markers for different renal or other organ system effects in the nonclinical and clinical space were outcomes of a consortium, alliance, or partnership.<sup>6</sup> From the list of Current Biomarker Qualification Submissions that are under review or undergoing consultation and advice, 15 of 19 submissions (~80%) are from consortia or partnerships.<sup>6</sup> Collaborative efforts create a unique opportunity for the qualification of DILI biomarkers by allowing investigators to gather rare clinical samples and combine data on specific phenotypes. There are potential future benefits for all participants of data pooling in an appropriate space to qualify DILI biomarkers.

The sections that follow present recommendations for obtaining and storing high-quality patient samples to fulfill the three critical conditions needed for biomarker qualification. Biomarkers that show the most promise to fill the gaps in the diagnosis and management of DILI in clinical development trials are also

discussed. While biomarkers for use in clinical development trials are the focus of this white paper, it is recognized that there is a need for new biomarkers for the management of DILI in clinical practice. Clinical practice is outside of the scope of this white paper, although there is an opportunity for qualified biomarkers for clinical development trials to progress to diagnostic application in clinical practice. Although current DILI biomarker explorations generally focus on hepatocellular injury and thus are a good model for qualification efforts, all forms of DILI would benefit from robust biomarker sample collection.

#### BEST PRACTICES FOR BIOSPECIMEN AND DATA COLLECTION

##### Collecting clinical samples for future research

Biobanking has revolutionized medical research in the ability to address unrealized questions through the evaluation of new biomarkers with the potential to facilitate precision medicine. For example, banked samples may be used to investigate common drug development challenges, such as unexplained lack of efficacy, placebo effects, or adverse events that were not predicted preclinically. Specific to DILI, biobanking could aid in the discovery of new biomarkers, particularly if heightened DILI risk associated with a study drug has not been identified early in clinical development. Biobanking is more common in clinical trials, but samples collected in a postmarket setting are also valuable. Typically, a much larger population will be exposed to the drug after it is marketed; thus there is potential to collect samples from larger numbers of individuals that will develop idiosyncratic DILI in this setting.

Nonetheless, biobanking also comes with challenges, specifically the cost of storage, distribution, harmonization of sample collection, and quality control procedures.<sup>7–9</sup> The logistics of sample collection also entail the cost of management, shipment, data transfers associated with the samples, and linking of metadata, which can vary depending on the type of sample collected. One possible solution to these challenges is offered in the model of Research Ready Hospitals<sup>10</sup> that proposes a centralized governing body to manage specimen collection at participating hospitals and clinical trial sites. This model has been successful for Acute Stroke Ready Hospitals through the certification program managed by the Joint Commission (<https://www.jointcommission.org/>). However, it may be more difficult to implement this model for rare adverse events, such as DILI, due to the need for continued training of medical staff on consistent procedures. It is nevertheless an interesting perspective on tackling the deterrents to biobanking.

Additional challenges remain when analyzing banked biospecimens. Incidental findings in biomarker results may also need to be communicated to patients to fulfill ethical responsibilities. Progress has been made through the release of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) M18 guidance,<sup>11,12</sup> which recommends using explicit informed consent forms that are signed prior to biospecimen donation and addresses the need for possible future data mining.

Despite these challenges, creating an environment in drug development where biospecimens are routinely and systematically collected and stored, regardless of therapeutic area or predetermined DILI risk, can facilitate cross-organizational biomarker qualification efforts.

### Recommendations for informed consent

To facilitate future biospecimen research, samples obtained from patients in clinical trials and postmarket studies must be appropriately consented. It is important to prospectively design consent forms to permit specimen collection and future biomarker analyses, as re-consenting of clinical trial participants is difficult and costly. Informed consent for biomarker evaluation needs to include essential key features that are necessary to permit effective biomarker evaluation. Published guidances are available, and thus the following is intended only as a general overview.<sup>13–15</sup>

Samples for biomarker analysis should be consented both for a broad range of analyses, including those related to adverse events and DILI, and a long storage duration. Even if a fixed set of biomarkers to be evaluated is known at the time of collection, as DILI biomarker research evolves it may become necessary to reanalyze samples later using alternate methods or for new biomarkers. Moreover, the breadth of the consent should ideally not limit the use of the samples or data to one investigational compound, indication, or sponsor, so that samples and data may be pooled for future biomarker discovery or qualification.

In addition, while investigators and participants are typically allowed access to their study records and results, it is recommended that data for nonqualified biomarkers are not returned to investigators or trial participants. Exploratory DILI biomarkers have not yet been qualified for clinical use or critically assessed for their predictive values of DILI in defined treatment populations. Also, concentrations of exploratory DILI biomarkers may have been measured using insufficiently validated assays. Performing analyses in laboratories compliant with Clinical Laboratory Improvement Amendments or similar regulations is warranted for definitive diagnosis. As such, certain biomarker data provided to investigators or participants may be incorrectly interpreted to have medical significance or be medically actionable.

For global trials, the informed consent for biospecimens, especially for pharmacogenetic blood samples, will need to account for the different country-specific regulations and local laws. As an example, the Revised Common Rule regulated by the US Code of Federal Regulations focuses on protection of patient privacy, whereas some states impose supplemental restrictions, mostly specific to genetic testing and potential discrimination in the workplace or health insurance.<sup>16</sup> Therefore, a well-defined process that can link each sample to the version of informed consent that was signed is needed. Also, a universal consent form that is readily accessible and vetted across multiple drug developers through a collaborative effort would benefit the field.

### When and how often should samples be collected

The schedule of sample collection is critical to the success of DILI biomarker data interpretation. Sometimes, preclinical evidence or a class effect that may suggest increased risk for a specific

mechanism of DILI already exists and guides the timing of sample collection. However, such information does not always exist prior to clinical trial conduct. Typically, genomic DNA samples are collected once at the start of the clinical trial. With appropriate informed consent language, it may be possible to leverage other samples (i.e., pharmacokinetic or predefined biomarkers) for future assessments if the matrix is compatible with the assay method. To define the frequency and spread of timepoints for collection of other biospecimens, it is important to consider temporal differences observed between different mechanisms of DILI while balancing operational aspects, such as feasibility related to the specific tissue or sample being collected. Depending on the mechanism of injury, the onset and recovery times of DILI may be different, presenting as an acute, chronic, or a delayed effect, thus potentially having different times for peak or trough levels of biomarkers. Onset of DILI typically varies between 5 days and 3 months after start of a medication but could also occur earlier, e.g., 24–72 hours in case of hypersensitivity reactions, particularly upon reexposure (sulfonamides, macrolide antibiotics). Furthermore, there are drugs that cause liver injury late, i.e., 3–12 months after start of administration (isoniazid, flutamide), and others for which the liver injury may become clinically evident after years of use (minocycline, amiodarone, nitrofurantoin, tocilizumab).<sup>17–19</sup> The recovery time can also vary. After an overdose event, acetaminophen-induced acute liver injury often resolves relatively quickly. In contrast, idiosyncratic DILI only starts resolving during the first week ceasing administration, reaching complete resolution after 2–3 months.

The time course and temporal nature of different biomarkers could also vary depending on the intracellular location or mechanism of injury responsible for their release and the mechanism of clearance. Some biomarkers released by leakage from the cell may exhibit early peaks and/or resolution over hours and days (e.g.,  $\alpha$ -glutathione *S*-transferase).<sup>20</sup> Others that reflect hepatocellular necrosis (e.g., ALT or aspartate aminotransferase), inflammatory or immunologic mechanisms (e.g., macrophage colony stimulating factor 1 receptor (MCSFR1)) may reach peak concentrations over a longer period of time. The resolution of different biomarkers could also vary greatly, impacting the optimal timepoints for collection. For example, glutamate dehydrogenase (GLDH) elevations rapidly return to normal levels following acetaminophen injury, while ALT levels can remain elevated over longer periods of time.<sup>21</sup>

The schedule of biomarker sample collection may be somewhat flexible. However, collection would ideally be synchronized with the timepoints designated for clinical data to facilitate mechanistic interpretation of the measured biomarkers in relation to clinical findings. Establishing the timepoints for biomarker sample collection may also depend on the phase of the clinical trial, as data are cumulatively gathered, as well as an objective to align with other study design requirements. In addition, some trials confine participants, while in others the participants can leave and only return to the clinic for defined protocol assessments. Therefore, the timepoints for sample collection may need to be designed around scheduled on-site visits and aligned with collection times for standard liver tests.

As stated above, accounting for the differences in presentation of liver injury, a proposed schedule of sample collection should be designed to enhance consistency of the collection timepoints between study subjects with DILI, relative to the onset of their liver injuries, and cover a combination of early and late timepoints following baseline. The earlier time points would capture direct dose-dependent hepatocellular damage, and later time points may be helpful for delayed onset idiosyncratic reactions which may require activation of an immunological component following the initial drug exposure. An example of a protocol of scheduled candidate biomarker collections when liver injury is not an *a priori* concern for exploring early onset DILI can include the following: pre-dose (day 1) and post-initial dose, day 3 (if possible, in phase I trials), week 1, week 2, week 3, week 4, and week 8. In instances when there is an observed clinical DILI event, this schedule of collection can be expanded to also include sampling times marked from the onset of liver injury. In addition, to adequately capture chronic and/or delayed onset patterns of liver injury, samples may be collected at 3, 6, and 12 months after the start of drug treatment depending on the follow-up design of the trial. If DILI is a known risk going into a clinical trial, the above timing should be modified case by case depending on existing data that support a specific mechanism. It is critically important to collect samples in this fashion from all cases of liver injury, including those that will emerge as causally associated with an etiology other than exposure to the study drug. These samples will provide important controls for the characterization of candidate DILI biomarkers, as described below.

#### **Best practices for collection and storage of samples**

Proper collection and storage of samples are critical aspects that impact the integrity and success of any biomarker initiative.<sup>22,23</sup> It is important that the overall strategy and standard operating procedures for the collection, aliquoting, transport, storage, and/or retrieval are put in place before the conduct of the study to ensure consistency.

**Sample collection.** Technical standardization of sample collection and processing is needed to minimize pre-analytical variability and should be tailored for the biomarker of interest as well as the matrix the biomarker is collected in. The use of anticoagulants for blood processing and the addition of stabilizers should be incorporated in sample collection to reduce potential interference in the biomarker assay(s). Aliquoting samples can reduce sample degradation and variability due to repeat freeze/thaw cycles and should be performed at a qualified facility prior to initial freezing whenever possible. If stability is a concern, for example labile or light/heat sensitive molecules, or is unknown at the time of collection, the recommendation is to aliquot the sample at the clinical site before freezing and shipping to central laboratory.

**Sample storage.** Samples should ideally be maintained under storage conditions that are consistent and secure with a complete audit trail, preferably in repositories designed with monitored temperature, humidity, and lighting conditions with safeguards in place. Containers used to store the samples should be chosen based on the kind of tissue, solvents used, duration of storage,

light sensitivity, and the need to adapt to automated platforms. Container labels should be resistant to temperature changes, solvents, and handling. Temperature and freezer condition ranges should be predefined for optimal shelf life for the specific type of biospecimens. Plasma samples stored at  $-70^{\circ}\text{C}$  without any freeze/thaw cycles were demonstrated to retain at least 4 years' shelf life with minimal impact on the plasma proteome measured by mass spectrometry.<sup>24</sup> Inventories of accurate records of the number, location, and storage conditions as well as ongoing retrieval and use for the samples need to be coupled with information management systems set up to ensure tracking and managing of these data in real time. Records of periodic quality control assessments such as monitoring of freezers and other applicable equipment and tests for the validation of long-term stability of biomarkers should be maintained to ensure sample quality. Due to the potential biohazardous nature of the samples, responsible disposal needs to be carefully documented as well. Database linkages of individual biomarker samples to the corresponding case-level clinical and diagnostic findings of the liver injury should be implemented, as described below.

#### **Data to be collected along with clinical samples**

Well-defined phenotypes are necessary to establish a definitive diagnosis and an adequate causality assessment of suspected cases of DILI but are also required for biomarker qualification to reduce variability that may be caused by diverse physiological mechanisms.<sup>25</sup> It is important that samples are obtained from all study subjects with significant liver injury, irrespective of the etiology. To facilitate an analysis of potential liver safety biomarkers, samples should be individually linked to all the collected case-level clinical data that support and characterize a diagnosis of DILI associated with the study drug or of a liver injury caused by another etiology. The use of a unique identifier will also be required to link a sample with the corresponding clinical data. For analysis of liver safety biomarkers, a unique participant identifier must be created to link the biomarker results with the clinical information for each individual patient. Integrating data across different sources is critical for success and requires creation of common data standards.<sup>26</sup> The Clinical Data Interchange Standards Consortium has developed a number of data standards for clinical research,<sup>27,28</sup> which are now available and may facilitate the ability to link biomarker samples with corresponding clinical data.

Collection of a complete set of clinical data is critical for the biomarker analysis. There are recommendations for the optimal data set that is needed for evaluation of DILI cases in clinical trials.<sup>29</sup> At a minimum, clinical data sets that are needed to evaluate liver safety biomarkers should include demographic information, start/stop dates of study drug, concomitant medications, underlying medical conditions as well as previous liver disease or metabolic syndrome, and clinical signs and symptoms of DILI, along with symptom onset date and laboratory evaluation. Laboratory tests required for the evaluation of DILI include standard liver tests longitudinally, hematology/coagulation, viral hepatitis serology, autoimmune serology, and liver imaging results.<sup>25</sup> Much of these data are more easily obtained in well-controlled clinical trials, compared with postmarket settings. In addition, validated scales of liver dysfunction can be utilized as part of data collection in certain

clinical trial settings. These include the Child Pugh Turcotte classification,<sup>30</sup> METAVIR scoring system,<sup>31</sup> model for end-stage liver disease score,<sup>32</sup> and pediatric end-stage liver disease model.<sup>33</sup>

Nonetheless, because the size of the postmarket treatment population exposed to the suspect drug typically is much larger than the clinical study population, rare forms of severe idiosyncratic DILI are more likely to occur after the initiation of marketing. For this reason, study protocols that incorporate biomarker collection in a postmarket setting are also encouraged, as these may yield useful data about candidate biomarkers. Postmarket sample collection that conforms to regulatory standards and a requirement for informed consent provides an opportunity to access the broad population.<sup>34</sup> To facilitate an assessment of DILI risk, it is recommended to collect as much clinical and diagnostic data as possible during the sample collection of postmarket liver injury cases, even if the timing of the collection may be suboptimal with reference to the times of actual onset and peak of liver injury.

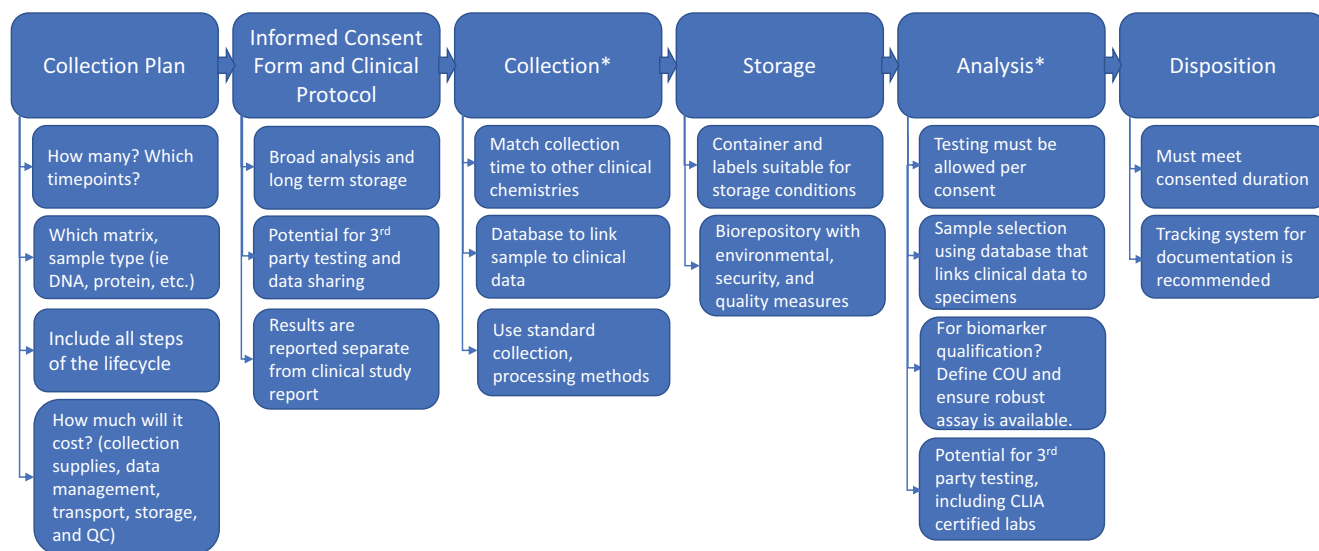
The components and considerations in the life cycle of sample collection to support the qualification of potential DILI biomarkers are summarized in **Figure 1**.

## STANDARD LIVER TESTS

### Clinical biomarkers routinely used to detect liver injury

In current clinical practice, there is a limited number of serum biomarkers used routinely to monitor the health of the liver (see **Table 1**).

Although these biomarkers have been used for many decades and are very useful in clinically assessing and managing patients with liver injury, they have shortcomings. ALT, a highly sensitive biomarker that is elevated in the serum during hepatocellular injury, can also be present due to several other conditions, including (i) glucocorticoid-related enzyme induction,<sup>35</sup> (ii) decreased clearance secondary to impaired Kupffer cell function,<sup>36</sup> (iii) damage to other tissues, such as muscle,<sup>37</sup> and (iv) intense exercise.<sup>38</sup> Elevations of total bilirubin (TBILI) are observed for liver injuries caused by other diseases. Gilbert's syndrome is a fairly common genetic condition of impaired bilirubin conjugation that is considered benign and does not generally need treatment, but drugs whose disposition depends on the uridine 5'-diphosphoglucuronosyltransferase (UGT) pathway may have aberrant pharmacokinetics and pharmacodynamics in patients with Gilbert's



**Figure 1** Life cycle of prospective sample collection for the qualification of biomarkers. CLIA, Clinical Laboratory Improvement Amendments; COU, context of use; QC, quality control. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**Table 1** Current serum liver biomarkers used in clinical practice

Biomarker	Tissue specificity	Cellular localization	Liver damage detected
Alanine aminotransferase (ALT)	Multiple tissues	Cytoplasmic	Hepatocellular injury
Aspartate aminotransferase (AST)	Multiple tissues	Cytoplasmic & mitochondrial	Hepatocellular injury
Total bilirubin (TBIL)	Liver	N/A	Cholestasis & hepatobiliary injury, hepatocellular injury in association with ALT/AST and a measure of liver function
Alkaline phosphatase (ALP)	Multiple tissues	Cell membrane	Cholestasis & hepatobiliary injury
Gamma-glutamyl transferase (GGT)	Kidney > Liver, Pancreas	Cell membrane	Cholestasis & hepatobiliary injury

N/A, not applicable.

syndrome.<sup>39</sup> When DILI is the cause, one major shortcoming of elevated TBILI is poor sensitivity; by the time elevations in circulating TBILI are detected in conjunction with ALT elevations, the patient may have an advanced stage of drug-induced liver damage. In addition, TBILI elevations can also occur in the setting of hemolysis as well as with inhibition of bilirubin transporters and the UGT1A1 enzyme. To evaluate potential DILI, the standard liver tests are used in the evaluation of Hy's Law.<sup>40</sup> Thus, the specificity of serum aminotransferase elevations and the sensitivity of changing bilirubin levels for DILI are both limited. In addition, other causes of these standard biomarker increases must be ruled out before attributing them to DILI.

### **Systematic assessment of standard liver safety biomarkers to assist in sample selection for qualification**

Qualification of novel biomarkers often begins with nonclinical studies to investigate feasibility of use, as well as mechanistic studies in animals to link the putative biomarker with specific liver pathology. Clinical outcome measures may be rooted in binary or continuous functions. Based on the predicted incidence of DILI in a clinical treatment population, clinical qualification can be undertaken either with randomized controlled trials or case-control studies in which at least two populations are compared by grouping the biomarker concentrations in each population and using statistical analyses to show a significant difference. With different types of outcome measures and predicted rates of DILI, utilization of an appropriate set of statistical approaches for assessing the association between a biomarker and an outcome measure is vitally important.<sup>41</sup> For a binary outcome measure, results can be evaluated using positive and negative predictive values or by evaluating receiver operating characteristic curves. In clinical trials, before this process can begin, the populations that are being compared need to be clearly assigned a phenotype, which requires a systematic exploration of the existing clinical data. The use of graphics, as pioneered since 2008 by US Food and Drug Administration (FDA) eDISH (Evaluation of Drug Induced Serious Hepatotoxicity) concept, has been proven highly efficacious in this context.<sup>42</sup> The core element of the eDISH method is a log/log scatter plot of peak TBILI vs. peak ALT values for all patients in a clinical trial, designated with specified symbols by treatment group, allowing drilldown from each individual data point to its corresponding liver chemistry time course graph associated with a patient narrative that contains sufficient clinical and diagnostic information required to both clinically characterize the case and assess its causality. Advanced techniques have also been described. For example, modified eDISH (mDISH) incorporates statistical outlier detection methods to define population-specific signal thresholds as well as changes from individual baselines.<sup>43,44</sup> Shift plots, liver chemistry time profiles, and comprehensive patient profiles, including lab, adverse event, and concomitant medication data, have also been instrumental in in-depth analysis of available data, hypotheses generation, and causality assessment. The elements described above have been incorporated into a comprehensive systematic workflow using interactive graphics software that can be adapted and customized to specific project needs.<sup>45</sup>

Systematic use of the workflow ensures optimal information is utilized from standard liver biomarker data and additional relevant clinical data in order to define a subject as a DILI case, as well as the specific phenotype that best describes the context of use (COU)<sup>46</sup> for the candidate biomarker that is being studied. It cannot be over-emphasized that efficient database linkages that bridge case-level clinical data with their corresponding traditional liver test biomarker data, and in some cases exploratory DILI biomarker data, will strengthen the biomarker qualification process by facilitating the procedure that can be used to select samples based on the defined phenotype. Current readily available workflows and software programs lack the ability to link the clinical data to the location and consent information of the biospecimens that are banked from those subjects. One consideration is to link samples to an eDISH plot or other statistical and graphical methodologies.<sup>47</sup>

It is recommended that those working in the DILI field make efforts to enhance the graphical tools currently available so that they incorporate the biospecimens into the case-level clinical data representation to facilitate progress in the study and qualification of new biomarkers.

### **BIOMARKER QUALIFICATION**

Avenues to biomarker qualification relevant to several organ systems including the liver are outlined in guidance documents from the FDA<sup>48</sup> and the EMA (European Medicines Agency)<sup>49,50</sup> and have been actively pursued by different groups, including the Critical Path Institute (C-Path) Predictive Safety Testing Consortium (PSTC), a US-based public-private partnership (PPP), and the Innovative Medicines Initiative's (IMI's) Safer and Faster Evidenced-based Translation (SAFE-T) consortium, a European-based PPP. Additionally, an evidentiary standards framework has been proposed for biomarker qualification.<sup>51</sup> The TransBioLine (Translational Safety Biomarker Pipeline), a new undertaking by the IMI, has initiated a research proposal to develop and qualify biomarkers of injury for several organs. In addition, collaborative efforts through organizations and PPPs that analyze samples collected over a wide range of therapeutic areas and causal drugs such as the US Drug-Induced Liver Injury Network (DILIN) and academic institutions plan to generate DILI biomarker data to support qualification efforts. The Acute Liver Failure Study Group is a network that has studied candidate biomarkers in patients with acute liver failure<sup>52</sup> that could potentially be further evaluated in a prospective fashion in clinical trials. The pooling and sharing of data that would be accumulated across different networks and research programs, as described above, has the potential to greatly accelerate progress in the qualification of DILI biomarkers. Collaboration between research groups is especially important because the qualification of these biomarkers will require the accumulation of sufficient numbers of DILI and control subject serum and urine biospecimens that are linked to well-annotated databases.<sup>53</sup>

An important aspect of biomarker qualification is assay validation. Laboratories need robust and reliable assays that require low sample volume and provide reproducible and accurate data. The assay performance should be evaluated and optimized for robustness before biomarker qualification measurements begin. Assay

parameters should also be standardized to minimize variability across laboratories. Briefly, there are different levels of validation that are recommended based upon the intended use of the biomarker data, and therefore a fit-for-purpose approach is recommended. For example, if a biomarker will be used for regulatory decision making, then full assay validation is required.<sup>54–56</sup> The framework includes (i) describing the drug development need, (ii) defining COU, (iii) considering potential benefits if the biomarker is qualified, and (iv) considering potential risks if the biomarker is used in a clinical development program.

## POTENTIAL DILI BIOMARKERS

### COU for potential DILI biomarkers

The PSTC and the former SAFE-T consortium have been leading efforts to qualify potential DILI biomarkers.<sup>57,58</sup> In SAFE-T clinical DILI studies, biomarkers were rated according to their performance for three separate COUs. These COUs were (i) to provide additional information beyond the diagnostic value of ALT and TBILI according to the pathophysiological mechanisms of hepatocyte necrosis, apoptosis, or immune activation; (ii) to anticipate a risk for progression of hepatocellular injury to severe DILI in patients in whom an initial DILI diagnosis has been established based on elevations of the standard marker ALT alone or in combination with TBILI; and (iii) for the assessment of suspected intrinsic liver injury by careful temporal monitoring of a potentially hepatotoxic drug before elevation of the standard marker of ALT. Based on preliminary data, a number of biomarkers have been identified for each of these COUs that could be incorporated into clinical trials.<sup>58</sup>

In 2016 the FDA issued a letter of support for DILI biomarkers.<sup>59</sup> A letter of support briefly describes the agency's opinions based on a submitted briefing book on the potential value of a biomarker and encourages further evaluation. The FDA has encouraged the further development and exploratory use of the biomarkers total cytokeratin 18 (K18), total and hyperacetylated high mobility group box 1 (HMGB1), osteopontin (OPN), and MCSFR1 alone or in combination as soluble monitoring biomarkers to assess the risk of progression of DILI in patients in whom an initial DILI diagnosis has been established based on elevations of the standard biomarkers ALT alone or in combination with TBILI as a clinical safety assessment in clinical trials in a drug development context.<sup>59</sup> In addition, the FDA has indicated that the biomarkers microRNA-122 (miR-122) and GLDH could be studied further as biomarkers of liver-specific injury based on their performance in patients with acute DILI, including patients with DILI due to acetaminophen overdose, compared with controls without liver injury.

### Defining reference values for potential DILI biomarkers

Well-defined reference ranges that correlate with healthy livers, diseased livers, and DILI, or confounding conditions in which the biomarker may respond if not completely liver specific need to be established. Normal reference ranges in a healthy volunteer population were measured in a collaboration between the PSTC, SAFE-T, and DILIN. The biomarkers evaluated included miR-122, K18, caspase-cleaved cytokeratin 18 (ccK18), GLDH,

$\alpha$ -glutathione *S*-transferase, alpha-fetoprotein, arginase-1, OPN, sorbitol dehydrogenase, fatty acid binding protein, cadherin-5, MCSFR, paraoxonase 1 (PON1 normalized to prothrombin protein), and leukocyte cell-derived chemotaxin-2. In general, individual variabilities based on coefficients of variation were low. However, there was high interindividual variability for miR-122 in the two different cohorts studied (~91% and ~213%) and high intraindividual variability of ~94% in one of these cohorts.<sup>60</sup>

Potential biomarkers of hepatic injury were evaluated in healthy human volunteers from serum samples collected from 550 healthy volunteers at the Pfizer Clinical Research Unit and the University of Michigan.<sup>61</sup> GLDH, malate dehydrogenase, purine nucleoside phosphorylase, and PON1 were evaluated in addition to ALT. GLDH and malate dehydrogenase levels were not affected by age or gender and showed good correlation to ALT compared with PON1 and purine nucleoside phosphorylase. These studies underscore the value of evaluating potential DILI biomarkers in healthy human cohorts as a necessary step in determining whether there may be a diagnostic value of these markers for detecting liver injury.

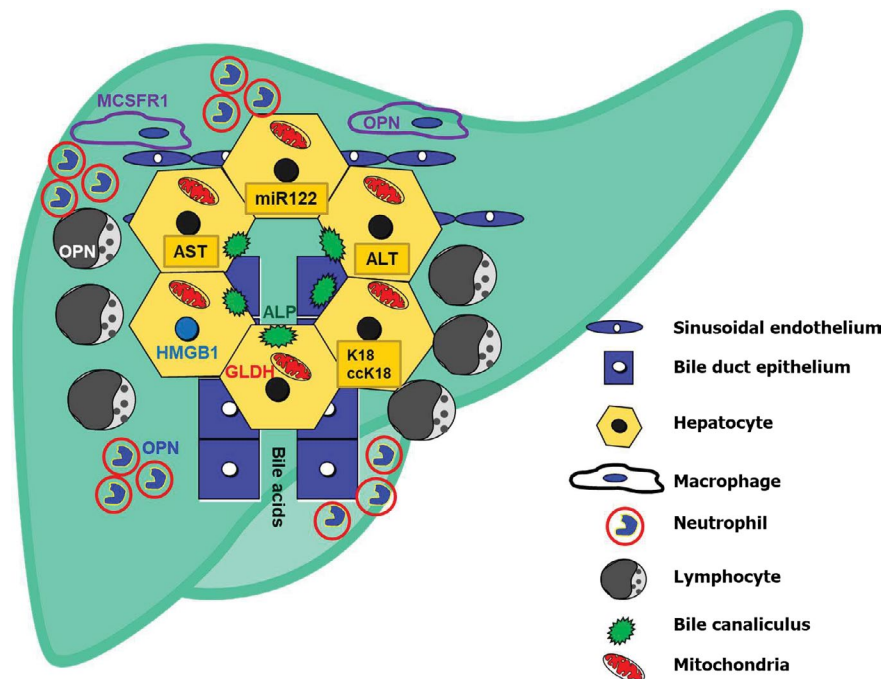
### Emerging biomarkers

Additional DILI biomarkers are under investigation to address some of the shortcomings of the established biomarkers. The focus for potential biomarkers of liver injury has remained on systemic moieties that may be evaluated in serum or plasma samples (Figure 2). These have been preferred due to their ease of accessibility to samples to monitor the patient and relative ease of assay development.

A well-defined COU is critical for this process. As previously discussed, support has been given by the FDA for a biomarker that can predict whether a patient will progress to severe liver injury after initial diagnosis of DILI. Such a biomarker would provide great benefit for human health by allowing clinicians to make informed decisions on treatment options. However, prediction of mechanistic pathways for cell death could also guide treatment decisions. Necrotic DILI may present itself differently from apoptotic or immune-mediated DILI, thus allowing a clinician to assess the outcomes of severity or longevity of the injury. For example, apoptotic DILI correlated with ALT elevations could result in adaptation to the hepatotoxic insult that is causing an ALT rise, and the offending medication can be safely continued. In contrast, necrotic DILI could indicate a more clinically serious state of injury and therefore require immediate termination of treatment with the offending drug. Another potential COU for a biomarker is to differentiate underlying or subclinical liver disease from DILI.

Based on existing data and the proposed COUs, a number of potential DILI biomarkers have been identified that show promise with measurement by robust assays (Table 2). However, generation of additional data is needed to further the qualification process, which can be gathered through analysis using samples from well-controlled biorepositories with predefined phenotypic assignment or through prospective incorporation into clinical trials.

GLDH is a biomarker of liver injury with increased predictive power over ALT to detect hepatic injury<sup>58,61,62</sup> and demonstrates improved specificity to the liver over ALT, particularly in differentiating



**Figure 2** A schematic representation of the hepatic source of emerging biomarkers including potential inflammatory cell infiltration during the course of drug induced liver injury (DILI). The biomarker time course and temporal changes in the circulation depend on the biomarker location within the liver and on the mechanistic basis of hepatocellular injury. While ALT, AST, GLDH, miR-122, and K18 (and its caspase-cleaved fragment ccK18) originate from within the hepatocytes, alkaline phosphatase and bile acids originate from pathophysiological changes within bile duct epithelium. Within the hepatocytes, ALT and AST are cytosolic, GLDH is mitochondrial, and HMGB1 is nuclear in location. MCSFR1 originates from macrophages and OPN from infiltrating mononuclear cells such as macrophages and lymphocytes. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GLDH, glutamate dehydrogenase; HMGB1, high mobility group box protein 1; K18, total cytokeratin 18; MCSFR1, macrophage colony stimulating factor receptor 1; miR-122, microRNA-122; OPN, osteopontin.

from muscle injury. In the healthy population cohort at the University of Michigan, there were no differences in levels based on age or gender.<sup>61</sup> GLDH also resulted in little intrasubject and intersubject individual variability in the PSTC healthy cohort.<sup>60</sup> GLDH, however, might add value only in the context of increases of other liver enzymes, and an isolated increase of GLDH might only indicate a biochemical abnormality without clinical relevance. Notably, transient elevations of GLDH levels may occur in the context of common bile duct stone passage or as a consequence of circulatory disturbances leading to centrilobular hypoxia in the liver like acute right heart congestion.<sup>63</sup> Furthermore, increasing GLDH values were observed in healthy subjects treated with medicines not associated with clinically important liver injury (e.g., cholestyramine).<sup>64</sup>

*HMGB1* has been evaluated as a damage-associated molecular pattern<sup>65</sup> related to inflammation<sup>66</sup> and has demonstrated increased levels with acetaminophen-induced liver injury.<sup>58,60,67</sup>

*Total K18* is widely expressed but is highly abundant in the liver. This protein is released into the circulation during hepatocellular injury. Protein is released as a full-length polypeptide (K18) or cleaved by proteases in the caspase family (ccK18). There is evidence that levels of K18 and ccK18 indicate the mechanism of cell death, i.e., necrosis and/or apoptosis.<sup>62,68,69</sup> K18 is significantly elevated in patients that die or require a liver transplant as a direct result of liver complications from an administered drug that causes DILI compared with spontaneous survivors.<sup>60</sup>

Overall, K18 and ccK18 resulted in little variability in the PSTC and SAFE-T healthy cohorts.<sup>60</sup>

*MCSFR1* has garnered some interest in the role of detecting immune-mediated liver injury.<sup>60</sup> This is particularly of interest for monitoring patients that are on immunotherapies that may compromise the immune tolerance of the liver.<sup>60</sup> *MCSFR1* demonstrated little variability in the SAFE-T healthy cohort, which was the only analyte in this study that was measured in plasma and not serum.<sup>58</sup>

Data from the ximelagatran biomarker discovery study suggest that *MCSFR1* is shed from macrophages during DILI.<sup>70</sup> In addition, in patients with acetaminophen-induced acute liver failure, a low serum level of *MCSFR1* was associated with increased mortality.<sup>71</sup>

*Total and individual bile acids* (conjugated and unconjugated) assessment may be used to evaluate the mechanistic basis for increases in ALT.<sup>58</sup> Individual bile acids may reflect inhibition of specific hepatic transporters (e.g., bile salt export pump)<sup>72</sup> or indicate bile duct hyperplasia in a nonclinical species. Certain individual bile acids were shown to differentiate subjects with acetaminophen overdose liver injury from both subjects with liver disease and healthy subjects.<sup>73</sup> In this study, age and sex had no impact on serum concentrations of bile acids. However, there was a significant increase in concentrations of four individual bile acids in those of Asian descent compared with other ethnicities. Translational gaps exist between humans and nonclinical species with regard to



**Table 2 Association of potential DILI biomarkers with context of use and mechanisms of liver injury**

Context of Use (COU)	Potential biomarker	Mechanism	Population variability	Assay	References
Mechanistic diagnostic to supplement ALT	GLDH	Necrosis	Low interindividual and intraindividual variability; not age or sex dependent	Enzyme activity assay	58,60–64
	HMGB1, total	Necrosis	High intraindividual variability	ELISA; commercially available	58,60,65–67
	ccK18	Apoptosis	Low interindividual and intraindividual variability	ELISA; commercially available	60,62,68,69
	MCSFR1	Immune-mediated	Low interindividual and intraindividual variability	Immunoassay	58,60,70,71
	Total and individual bile acids	Biliary injury/dysfunction	High interindividual and intraindividual variability	LC MS; routinely available	58,72,73
Predict severe outcome after DILI diagnosis	OPN	Hepatic inflammation and necrosis	Low interindividual and intraindividual variability	ELISA or immunoassay; not routinely available	58,60,74–76
	K18	Necrosis/Apoptosis	Low interindividual and intraindividual variability	ELISA; commercially available	60,62,68,69
	MCSFR1		See details above		
Early diagnostic before ALT elevations	GLDH		See details above		
	HMGB1, total				
	K18				
	ccK18				

ALT, alanine aminotransferase; ccK18, caspase-cleaved cytokeratin 18; DILI, drug-induced liver injury; GLDH, glutamate dehydrogenase; HMGB1, high mobility group box protein 1; K18, total cytokeratin 18; MCSFR1, macrophage colony stimulating factor receptor 1; OPN, osteopontin.

the changes in bile acids related to bile salt transporters that can be likely addressed with this biomarker.

OPN is a phosphoprotein produced in a variety of tissues including the liver and plays a key role in mediating hepatic inflammation and the migration of inflammatory and cancer cells.<sup>74</sup> Serum OPN levels in fulminant hepatic failure patients were higher than those of acute hepatitis patients without liver failure and healthy adults. OPN levels also correlate with the degree of hepatic necrosis in acute liver failure. There is some evidence that OPN is associated with liver repair due to activation of hepatic stem cells. OPN resulted in little variability in the SAFE-T healthy cohort.<sup>58</sup>

Elevated serum levels of OPN are detectable in patients with severe liver damage, and patients with high serum OPN levels have a significantly poorer prognosis compared with patients whose serum OPN levels were not elevated.<sup>75</sup> OPN has also recently been shown to be a potential predictor of death/transplant in DILI.<sup>60</sup> Plasma OPN levels are not specific for DILI and are also significantly elevated in patients with hepatitis, including acute hepatitis, chronic hepatitis, and fulminant hepatitis.<sup>76</sup>

**Proteomics.** It is noted that individual proteins have not yet been identified as promising DILI biomarkers. However, proteomics for discovery of new biomarkers is an important consideration when collecting and storing biospecimens. For example, hypothesis-generating mass spectrometric techniques for semiquantitative and targeted proteomics, as well as innovative panel technologies such as O-Link immuno-PCR based approaches, have unique

analytical properties that should be incorporated into a biospecimen collection plan.<sup>77</sup>

**Others.** Although some recently identified biomarkers for liver injury have generated a high level of interest, some have subsequently been shown to have important limitations. For example, there was early excitement for use of miR-122 as a specific and sensitive biomarker of liver injury.<sup>58,59,69</sup> However, recent data in healthy volunteers suggest that this biomarker has high interindividual and intraindividual variability, making the setting of normal reference ranges for the biomarker problematic.<sup>60</sup> In addition, miR-122 levels may also fluctuate with liver disease in the absence of liver injury.<sup>78</sup> An exhaustive list of recently investigated biomarkers may be found in **Table S1**.

While the focus of the above-mentioned biomarkers is on single analyte assays, the best performing ones may be used in combination or as a panel where each biomarker addresses certain mechanisms and/or locations of injury. Ultimately, assay(s) for multiple analytes may be developed to facilitate both DILI prediction and their routine use in a real-world setting.

#### WHAT IS THE ROLE OF GENETIC MARKERS IN PREDICTING DILI?

There is increasing evidence that certain genetic polymorphisms contribute to DILI risk. However, these genetic risk factors appear to be largely drug specific as, to date, no single genetic risk

**Table 3 Genetic associations with drug-induced liver injury that have been confirmed in additional patient cohorts**

Drug	HLA risk allele or gene	NPV	PPV	References
Amoxicillin-clavulanate (EU)	A*02:01, B*07:02, DRB1*15:01, DQA1*01:02, DQB1*06:02	0.99	0.011	80
Flucloxacillin	B*57:01	0.99	0.0012	80
Lapatinib	DQA1*02:01	0.99	0.031	80
Ticlopidine	A*33:03	0.98	0.17	80
Ximelagatran	DRB1*07:01	0.95	0.19	80
Isoniazid	NAT2	NA	NA	79

HLA, human leukocyte antigen; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

factor has been associated with DILI across multiple drugs or drug classes.

Although there are scattered reports of associations between genetic variants involved with drug disposition and cytokine release with DILI susceptibility, the only consistently reproducible association has been *NAT2* polymorphisms due to antituberculosis drugs in Asian populations.<sup>79</sup> To date, the only other variants found to be reproducible as significant risk factors involve human leukocyte antigen (HLA) alleles (Table 3).<sup>80</sup> This finding, together with specific clinical characteristics of latency to onset and more rapid recurrence on rechallenge, supports a role for the adaptive immune system in idiosyncratic DILI.<sup>81</sup>

Many of the identified HLA associations have good negative predictive value, but all have low positive predictive value for DILI caused by the suspect drug (Table 3). Thus, the vast majority of people who carry the identified HLA risk alleles will not develop DILI when treated with the implicated drug. To date, there has been only one attempt to introduce genetic testing for the management of DILI risk. This involved lumiracoxib, a cyclooxygenase-2 inhibitor withdrawn from worldwide markets due to rare but severe liver toxicity.<sup>82</sup> The attempt to remarket lumiracoxib by linking treatment to genetic screening<sup>83</sup> was unsuccessful. Consequently, the effectiveness of genotyping in order to reduce the risk of DILI due to lumiracoxib has not been determined. Nonetheless, in specific instances for which there is adequate study data, the FDA and the EMA have supported the use of established predictive genotyping to enhance treatment decisions in patient care.<sup>84,85</sup>

As genotyping becomes a more common clinical practice, it seems likely that even genotypes with only a high negative predictive value will be used in clinical practice and drug development. For example, the ability to identify a substantial patient subpopulation not at risk for DILI could change the net benefit for the drug in those patients. HLA genotyping could also help identify the most likely causative agent in a patient receiving multiple potentially hepatotoxic medications. For example, if a patient does not carry a known HLA risk allele for a suspect drug (Table 3), then it might reduce the likelihood that the drug is a cause of DILI.

Many of the DILI risk alleles identified to date have come from studies by the International Serious Adverse Event Consortium (<https://www.saeconsortium.org/>), Pro-Euro-DILI,<sup>86</sup> and DILIN (<http://www.dilin.org/>). DILIN's prospective study identifies, enrolls, and collects clinical data and biospecimens from patients who

experience clinically important liver injury due to marketed drugs. However, due to the rarity of severe DILI events, the number of cases due to any single drug with biospecimens collected both before and after the injury is observed has challenged biomarker discovery. A recommendation for the future will be for the pharmaceutical industry to partner with the above-mentioned consortia and DILI registries when newly approved drugs are shown to have unanticipated liver safety liabilities and where abnormalities in traditional or newer DILI biomarkers were observed in the clinical trials. Retrospective analyses using banked and properly consented genomic samples from these trials will be instrumental in detecting DILI risk alleles. This approach could lead to relatively rapid identification of genetic tests to improve DILI risk management. Alternatively, if the reported postmarketing patients with the liver events did not carry the identified risk allele(s), it might be less likely that the events were related to the drug.

## SURVEY RESULTS

Eleven member companies responded to a survey that was distributed to all 12 member companies of the IQ DILI consortium that were active at the time of the survey to provide insight into how samples are collected and stored and the availability of samples and data that can be used for DILI biomarker qualification.

Out of 11 companies, 10 (91%) collect and store biospecimens from clinical trials (82%) or other sources, such as academic institutions and noninterventional studies (18%). Nine companies responded to a question about sample types collected, and these include whole blood (22%), plasma (100%), serum (78%), urine (67%), tissue (0%), and DNA (44%); eight responded that these are stored frozen at  $-70^{\circ}\text{C}$  or lower.

Companies that would consider providing anonymized samples in a collaborative effort for exploring or qualifying new clinical liver toxicity biomarkers are just less than the majority at 40% (4/10), although 50% (5/10) of the companies that responded did not know if their company would consider providing samples. However, samples that are broadly consented for exploratory evaluations are available from three out of nine companies (33%). The main reason that samples are not available is that the samples were not broadly consented.

DILI biomarker data were retrospectively generated from these stored samples from five out of nine (56%) companies, including data for bile acids (both total and individual), GLDH, miR-122,

miR panel, K18, ccK18, total HMGB1, and cytokines. For the biomarker data that already exist, 4 out of 10 (40%) companies would consider contributing anonymized data to an industry consortium for exploring new DILI biomarkers, and 40% of responses did not know if data sharing would be considered.

Measurement of novel liver biomarkers other than ALT, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, TBILI, and international normalized ratio during clinical trials was performed by three companies (27%), although all three utilized these markers in study-specific investigations that are not routinely used. The biomarkers for specific situations were chosen based on proposed mechanism and included ferritin, GLDH, individual bile acids, miR-122, K18, and acetaminophen adducts. A reason was not provided for including ferritin. Serum ferritin has been reported to correlate with serum ALT, which is consistent with the view that serum ferritin is a marker of damaged cells.<sup>87</sup>

## SUMMARY

There is a need for additional clinical research to develop new DILI biomarkers for safety assessment. There are a number of high potential biomarkers for DILI that are currently considered to be exploratory by both the FDA and the EMA.<sup>59</sup> Among these, GLDH appears to be a strong candidate for future qualification as a biomarker for liver injury, irrespective of the etiology. With support of regulatory agencies and an unmet clinical need, there is a unique opportunity to collaborate and advance our understanding of the diagnosis, management, and prevention of DILI by driving forward the qualification of exploratory biomarkers.

Regardless of whether signals of hepatotoxicity appear in the preclinical stages or early stages of human studies when the hepatotoxic profile of a drug has not been fully assessed, or whether hepatotoxicity has already been identified for another drug in the same class as the study drug, strong consideration should be given to collecting and archiving biospecimens from study subjects with liver injury. Key takeaways related to collection of biospecimens and clinical data are summarized in **Table 4**. For other compounds of unknown risk, the low prevalence of idiosyncratic DILI may require the administration of a drug to large populations before it is known to be a hepatotoxicant. Therefore, it is important to archive as many clinical samples as possible, regardless of known status of hepatotoxicity or other organ toxicity. It is also critically important to systematically collect samples from subjects exposed to the study drug who do not develop liver injury during treatment with the study drug, as well as individuals with new liver injuries caused by etiologies other than exposure to the study drug. Together with samples collected from study subjects randomized to receive the placebo or comparator agent, these samples will be required as controls in the comprehensive analyses that will later be performed on the candidate DILI biomarker.

It is thought that a full understanding and appreciation of the potential value of DILI biomarkers will only be gained following the measurement of thousands of samples, and clinical trials are the best controlled opportunity to carry out this sample collection.<sup>88,89</sup> Biospecimens should be collected concurrently with standard clinical chemistry labs to facilitate adequate experimental design of biomarker research. Determination of the

## Table 4 Key takeaways

### Key takeaways

It is recommended that all sponsors:

- Collect, with broad and ethical consent, and properly store biospecimens in clinical trials following procedures that allow for qualification of biomarkers.
  - Different matrices (i.e., plasma, serum, and/or urine) and timepoints may vary depending on predicted DILI risk.
  - A minimal sample set is still recommended even if risk of DILI is not predicted.
  - Multiple types of biomarkers should be considered (i.e., RNA/miRNA, protein and small molecules, and/or genomic).
- Collect clinical data concurrent with biospecimen and store data in a manner that is easily linked to the biospecimen, using graphical tools if possible.
  - At a minimum, demographic information, start/stop dates of study drug, concomitant medications, underlying medical conditions, clinical signs and symptoms of DILI (i.e., jaundice, rash, and right upper abdominal pain), longitudinal standard liver tests, hematology/coagulation, viral hepatitis serology, autoimmune serology, and liver imaging results, when feasible.
  - The collection of clinical data of those that are treated and do not experience signs of liver injury, as well as those that are not treated but do experience signs of liver injury, is important.
- If DILI risk is predicted, collect data on emerging biomarkers, either during clinical trial development or *post hoc* study setting, to advance biomarker qualification efforts.
  - Emerging biomarkers: including but not limited to GLDH, total HMGB1, K18, ccK18, MCSFR1, bile acids, and OPN.
  - Panels of biomarkers should be explored that cover multiple mechanisms of DILI.
  - Emerging biomarker data from patients that do not qualify as experiencing true DILI should also be collected, including those that show signs of etiology of disease similar to DILI.

ccK18, caspase-cleaved cytokeratin 18; DILI, drug-induced liver injury; GLDH, glutamate dehydrogenase; HMGB1, high mobility group box protein 1; K18, total cytokeratin 18; MCSFR1, macrophage colony stimulating factor receptor 1; OPN, osteopontin.

optimal schedule for sample collection should consider timepoints before treatment, during treatment, and after cessation of study drug in all enrolled subjects. These samples should be properly and broadly consented and stored under adequate conditions for optimal stability.<sup>25</sup>

It is hoped that from information included in this paper, readers sense the importance of DILI biomarker qualification efforts and look to ways to contribute samples to these causes. It is only through collaboration that the thousands of necessary samples will be acquired to lead to better tools to evaluate new therapeutics and monitor the risk of DILI for all patients.

## SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website ([www.cpt-journal.com](http://www.cpt-journal.com)).

**Table S1.** Full list of recently investigated potential new liver biomarkers.

## ACKNOWLEDGMENTS

Debra Luffer-Atlas, PhD, Eli Lilly and Company; Frank Simutis, PhD, Bristol-Myers Squibb; Will Treem, MD, Takeda; Arie Regev, MD, Eli Lilly and Company; Lara Dimick-Santos, MD, US Food and Drug Administration; Bob Fontana, MD, University of Michigan; Naga Chalasani, MD, Indiana

University School of Medicine; Christopher Leptak, MD, PhD, US Food and Drug Administration; Aliza Thompson, MD, US Food and Drug Administration; IQ Secretariat (Jillian Brady, Maureen Hardwick, Maggie Liu, Lana Lyapustina, Alexis Myers).

**FUNDING**

No funding was received for this work.

**CONFLICT OF INTEREST**

The authors declare the following competing interests as full-time employees of respective companies or agencies: S.E.R. (Otsuka), M.I.A. (FDA), D. Bourdet (Theravance Biopharma), D. Brott (AstraZeneca), R.C. (Eshelman School of Pharmacy, University of North Carolina), A.D. (Genentech), D.K. (Sanofi), P.S. (Celgene), L.W.-B. (Otsuka), S.L. (Bayer AG), L.R. (Bristol-Myers Squibb), S.K.R. (Pfizer), and J.M. (Takeda).

M.M. is a part-time employee at University Hospital of Zurich, Switzerland, and an independent consultant, currently working for AstraZeneca and Novartis. A.M.S. is an employee of Drinker Biddle & Reath LLP, which serves as Secretariat to the IQ Consortium, including the IQ DILI affiliate. S.E.R., D. Brott, A.D., D.K., L.W.-B., M.M., S.E.R., and J.M. are employed by companies that are collaborators of the Critical Path Institute, including the Predictive Safety Testing Consortium. In addition, P.B.W. has financial interest in DILISym Services, Inc., which models biomarkers.

**DISCLAIMER**

The views expressed are those of the authors and do not necessarily represent the position of, nor imply endorsement from, the US Food and Drug Administration or the US Government.

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