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The Liver Toxicity Knowledge Base (LKTb) and Drug-Induced Liver Injury (DILI) Classification for Assessment of Human Liver Injury

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Abstract

Introduction: Drug-Induced Liver Injury (DILI) challenges drug development, clinical practice and drug safety regulation. The Liver Toxicity Knowledge Base (LTKB) provides essential data to support the study of DILI.

Areas Covered: The LTKB brings together diverse data sources that can be used to assess and predict DILI. Among the extensive information available, reference drug lists with annotated human DILI risk are of great value. The LTKB DILI classification describes DILI severity concern (most, less and no DILI-concern) determined by integrating FDA drug labeling, DILI severity score from the NIH LiverTox database, and other DILI classification schemes described in published literature. Overall, over 1000 drugs are described in at least one classification scheme, and around 750 drugs were flagged for some degree of DILI risk.

Expert Commentary: The LTKB provides a centralized repository of information for the study of DILI and the development of predictive models. DILI classification data in LTKB could be a useful resource to connect phenotype with molecular and mechanistic data for developing biomarkers, predictive models and assessing data from emerging technologies such as high-throughput screening, high-content screening and in *silico* methodologies. In coming years, streamlining the prediction process by including DILI predictive models for both DILI severity and also including DILI types in LTKB would enhance the identification of chemicals with the potential to cause DILI earlier in drug development and risk assessment.

Keywords: Drug-Induced Liver Injury (DILI), Drug Classification for DILI, Human Liver Injury, Liver Toxicity Knowledgebase (LTKB), Computational Toxicology

1. Introduction

Safety and toxicity is one of the main reasons for drug attrition during preclinical and clinical drug development [1, 2]. Current testing strategies are based on the ICH (*International Conference on Harmonization*) guidelines [3] and employ both rodent and non-rodent toxicology studies to limit and manage risk to human volunteers and patients. However, the current preclinical testing paradigm may not predict some organ toxicities [1] such as Drug-Induced Liver Injury (DILI). To date, DILI is one of the primary concerns in drug development and is also a frequent cause of regulatory actions like Black Box Warnings or denial in approval [4]. Many efforts have been made to improve the ability of preclinical work to predict clinical outcome, especially for DILI.

In Europe, progress has been made towards using alternative means [3, 5] under the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) to evaluate the toxicity of chemicals using both *in vitro* and *in silico* approaches [6]. The *in vitro* techniques mainly focus on the use of high-throughput screening methodologies to record cellular responses simultaneously for multiple chemicals whereas the *in silico* methods aim to predict toxic responses based mainly on structure-toxicity relationships. For both methods, a reference list with a large number of compounds is required that classifies chemicals for DILI risk as either 'potential to cause liver injury' or 'does not cause liver injury'. This reference list is fundamental to underpin predictive toxicity efforts along with machine learning techniques that can build a profile of DILI negative and DILI positive characteristics[7].

When high-throughput screening and *in silico* approaches are applied to a large number of drugs, they depend on these drugs being correctly annotated, in this case by a reliable DILI classification scheme to identify markers that can distinguish DILI positives from DILI negatives. However, categorizing DILI for any given drug is challenging and somewhat controversial; thus, it must be approached in a systematic way. Since DILI risk assessment is multifactorial, one helpful way to categorize DILI is according to the following characteristics:

- i) causality – does this drug cause DILI?
- ii) incidence – how many individuals are affected and
- iii) severity – the clinical impact for the patient ranging from transient liver enzyme elevations through to liver failure (requiring transplantation).

These three characteristics have to be considered together in order to identify an integrated potential DILI risk for a drug [8]. Currently, there are several DILI classification schemes

reported [7-13] . Some place much emphasis on incidence in the population but DILI is rare, occurring in only a very small number of patients. Thus, the results derived from incidence-driven DILI classification vary among investigators perhaps due to under-reporting. Others schemes include all three data types outlined above. For example, the Liver Toxicity Knowledge Base (LTKB) project has developed a comprehensive DILI classification scheme and the FDA Labeling information balances these three sets of information [13].

2. Liver Toxicity Knowledgebase (LTKB) Overview

The Liver Toxicity Knowledge Base (LTKB) has been developed at the US Food and Drug Administration's National Center for Toxicological Research (USFDA NCTR) to serve as a reference for drug development related research. Freely available for public use, LTKB is designed to assist regulators, researchers and clinicians with their DILI related inquiries. LTKB contains DILI classification lists as described previously, in addition to the physio-chemical profile for each drug. In practical terms, LTKB is a warehouse of data that can be used for understanding DILI related phenomena, building models to make predictions and for finding associations to make inferences from data [7, 8, 13].

Currently, LTKB includes in vitro and in vivo data from preclinical studies, emerging technology data such as gene expression profiles, in silico data such as physico-chemical properties and chemical structure descriptors and epidemiology data such as reported adverse events. These data can be depicted in different categories such as histopathology, cellular responses, toxicogenomics, drug properties, therapeutic uses/side effects and patient response (Figure 1). By providing comprehensive information at one location, LTKB provides the opportunity to understand the underlying biological complexity of the response to individual or groups of drugs. Since users can perform searches driven by their own requirements, output is tailored to the query posed and the results from the queries can be downloaded in spreadsheet format for further use. In practical terms, users interrogate LTKB by inserting their specific information in a text box for each field. Structure-related searches include similarity, exact match and flexible matches. Searches can also be refined and made more specific using multiple step queries. Over 1000 drugs have been incorporated into the database which can be found at <http://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/>

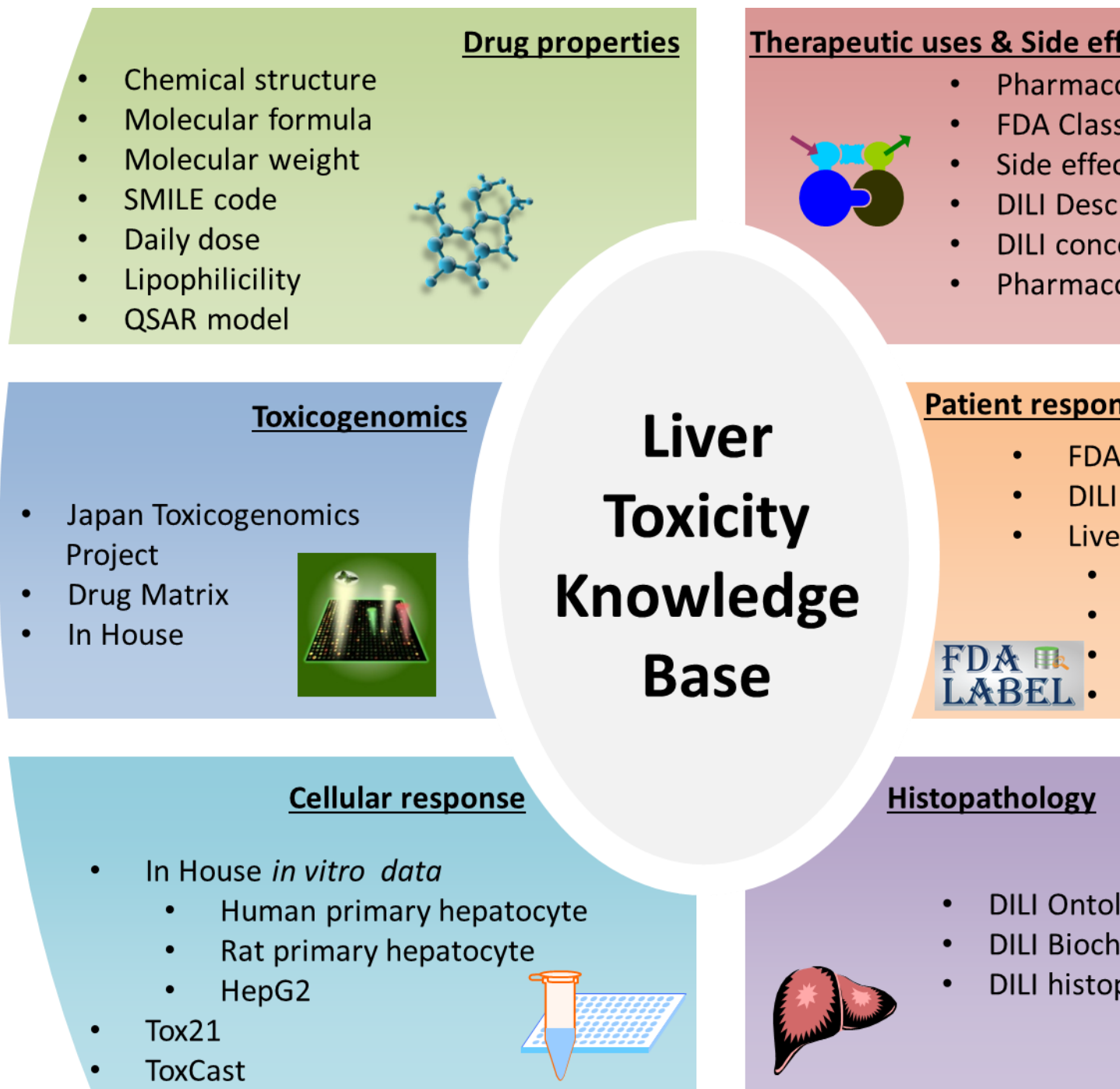


Figure 1. A summary of data in the Liver Toxicity Knowledge Base (LTKB). Data include DILI classification, drug-specific information such as chemical structures and physico-chemical properties, *in vitro* data and toxicogenomics data. The database is being linked to other publicly available databases such as ToxCast, Tox21 and LiverTox.

3. Resources available in LTKB

3.1 DILrank – the largest DILI classification dataset

The LTKB encompasses an approach to classify the DILI potential of drugs based on FDA drug labeling information [8]; a total of 1036 FDA drugs approved for human use with a single active molecule were included. The analysis focused just on drugs approved by the FDA before January 1st 2010 (Table 1) since it generally takes >5 years to collate the required post marketing safety information into the label to support a meaningful categorization [13]. Drug labeling-based hepatotoxicity information was extracted from three labeling categories: ‘boxed warning’ (commonly known as “Black Box Warning”) ‘warnings and precautions’ and ‘adverse reactions’. Originally, Chen et al [8] annotated 287 drugs (Table 1) by grouping them into three DILI categories (most-, less- and no-DILI-concern). The ‘most-DILI-concern drugs’ were in the categories: ‘withdrawn drugs due to hepatotoxicity’, drugs with ‘Black-Box Warning’ for hepatotoxicity and drugs with high severity noted for hepatotoxicity in ‘warnings and precautions’. Where ‘Warnings and Precautions’ indicated less DILI severity, the drug was classified as less-DILI-concern. ‘Adverse Reactions’ drug label was also classified as less-DILI-concern. In the absence of a hepatotoxicity related drug labeling, the drug was classified as no-DILI-concern [8]. More recently, this approach was revised and improved by incorporating DILI causality [13], which resulted in the DILrank dataset (Table 1). Specifically, DILrank contains verified (v) annotated as ‘Most-DILI-Concern’, ‘Less-DILI-Concern’ and ‘No-DILI-concern’. The fourth category for DILI concern was ‘Ambiguous DILI-concern’, for drugs where causality remains to be established [13].

3.2 DILrank versus LiverTox database

We compared the DILrank dataset with DILI information from LiverTox (livertox.nih.gov) severity scores. LiverTox [14] is a collaborative project between the National Institute of Health, National Institute of Diabetes Liver Disease Research Branch and Digestive and Kidney Diseases (NIDDK) and the Division of Specialized Information Services of the National Library of Medicine (NLM). LiverTox scores are assigned based on the patient’s clinical outcome. This severity level (scale of 1-5) was designed to address the clinical spectrum of DILI damage from mild ALT elevations (1 on the scale) through to death due to DILI (5 on the scale) (Table 1) [14].

As shown in Figure 2, the concordance between DILrank and LiverTox databases was consistent only for these drugs in two of the categories. Specifically, there was overlap for (i) Most-DILI-Concern (DILrank) and Fatal 5+ (LiverTox) and (ii) Less-DILI-Concern (DILrank) and Mild 1+

(LiverTox). For example, 25 out of 34 drugs (74%) causing Fatal DILI (severity level 5+) determined by LiverTox were also the Most-DILI-Concern drugs in DILIRank. Similarly, 24 out of 31 drugs (77%) with Mild DILI signal (severity level 1+) by LiverTox were the Less-DILI-Concern drugs by DILI Rank. Importantly, the drugs classified as safe by DILI Rank (i.e., No-DILI-Concern) have no DILI case reports collected by LiverTox. Although the general trend in DILI severity is apparent between the two databases, the one-to-one concordance was not statistically significant. Of note, DILIRank classifies drugs based on the DILI information gathered during drug development, clinical trials, approval and post-marketing studies whereas the LiverTox classification is based on clinical observation. In addition, the DILI pattern observed in the clinical setting described in LiverTox does not necessarily correspond to the severity defined by DILIRank. The difference in DILI classification between two databases therefore derives mainly from their source information.

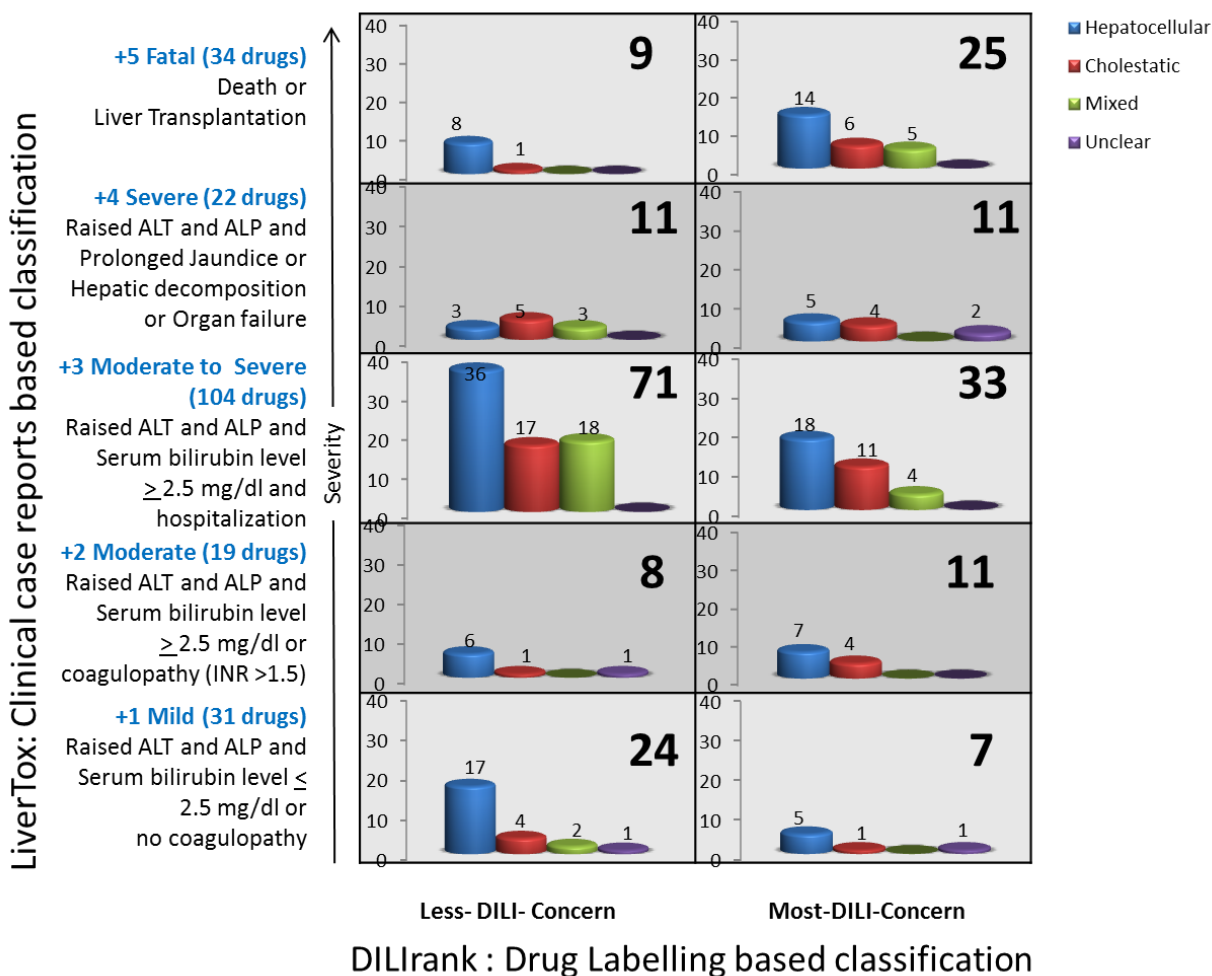


Figure 2: Comparison of DILLrank with LiverTox database. DILLrank was derived from a drug-centric DILI classification approach based on FDA drug labeling data whereas the LiverTox-based DILI classification is based on clinical observation. Each histogram represents a type of liver injury and also the number of drugs in that category reported to have that kind of injury. Hepatocellular, cholesteric, mixed and 'unclear' types of injury are represented depicted as blue, red, green and purple histograms respectively. No-DILI-Concern is not represented in this figure because there were no DILI related case reports collected by LiverTox for that category.

3.3 Other DILI classification methods in LTKB

Different reported DILI classifications tend to cover drug lists assembled from different sources, resulting in limited overlap in output. DILLrank contains the largest list of drugs annotated systematically based on drug labeling information. LiverTox, where DILI classification is determined by analyzing the case reports in the literature, is also a sizeable dataset. Besides these two datasets, LTKB also collates several other notable DILI classification datasets, summarized in Table 1 and described below.

Greene et al. [12] collated case reports for DILI and literature evidence for a total of 325 drugs and classified them as follows: 189 drugs with strong evidence for human hepatotoxicity, 50 drugs with weak evidence and 86 drugs with no evidence. In another published study, Suzuki et al. [9] consolidated DILI information for 225 drugs by collating DILI related information from major registries such as the Spanish DILI registry, the Swedish adverse reaction databases and the DILI network (DILIN) (USA). Finally, Xu et al. [11] collated 217 drugs for DILI causality based on case reports and literature information; 161 drugs were identified as DILI positive and 56 drugs as DILI negative. Guo et al. [10] collated 47 different drugs and classified them in three categories: 32 drugs were identified for severe hepatotoxicity (clear evidence in the literature that they endanger life). A further thirteen drugs demonstrated multiple case reports of hepatic injury and for 4 there were no reports of significant hepatotoxic damage.

Another interesting consideration is whether some therapeutic categories are overrepresented in DILI causation. To assess this, a statistical analysis of incidence according to therapeutic category (as obtained from DrugBank [15]) was conducted (Table 1). Using Fisher's exact test, the therapeutic categories that were significantly enriched with DILI related drugs (p-value ≤ 0.05) were identified. The results showed that antineoplastic agents are present in 6 out of 7 datasets, far more than other therapeutic categories.

Table 1. DILI classifications represented in LTKB

No.	DILI human classification in LTKB	DILI Evidence information	No. of Drugs*	Categories	Major Hepatotoxic Therapeutic categories**
1.	DILI Rank [12, 13]	FDA Drug Labelling and DILI causality evidence	1036	^v No-DILI-Concern (312) ^v Less-DILI-Concern (278) ^v Most-DILI-Concern (192) ^v Ambiguous-DILI-Concern (254)	Antineoplastic agents Antibacterial agents
2	LiverTox [11, 14]	Human DILI Case Reports	694	Non-DILI (98) Rare-DILI-Reported (69) DILI Literature Evidence (273) DILI Severity 1+ (40) 2+ (16) 3+ (128) 4+(25) 5+(42)	Antineoplastic agents
3	Greene et al. [12]	Literature evidence and DILI case reports	325	Human hepatotoxicity (189) Weak evidence (50) No Evidence (86)	NSAID's
4	DILI Benchmark Dataset[8]	FDA Drug Labeling	287	No-DILI-Concern (137) Less-DILI-Concern (85) Most-DILI-Concern (65)	Antineoplastic agents Antibacterial agents
5	Suzuki et al. [9]	DILI at Physician's Desk reference	287	General liver injury (157) Acute liver failure (87) withdrawn or suspension for DILI (43)	Antineoplastic agents, Antibacterial agents and Antihypertensive agents, NSAID's
6	Xu et al. [11]	Literature evidence and DILI case reports	217	DILI Positive (161) DILI Negative (56)	Antineoplastic agents NSAID's
7	Guo et al. [10]	5 major US drug compendia: American Hospital Formulary Service (AHFS), United States Pharmacopeia Drug Information (USPDI), Facts and Comparisons (F&C), Physicians' Desk Reference (PDR), and Clinical Pharmacology (CP)	86	No information (1) No significant liver damage (8) Significant liver injuries (24) Life-threatening hepatotoxicity (53)	Antineoplastic agents

* The number of drugs reported from each classification reflects drugs that were approved by the FDA before 2010

** The Therapeutic category is obtained from drug bank. The enrichment analysis is based on Fisher's exact test with p-value less than 0.05.

3.4 In vitro assay data in LTKB

In vitro model systems based on human liver-derived tissues such as tissue slices, cell lines or primary hepatocytes have been developed to help identify human DILI risk. Gustafsson et al [16] classified 104 drugs for human hepatotoxicity in human hepatocytes overexpressing cytochrome P450. Using human hepatocytes, Zhang et al [17] identified severe DILI-causing drugs using a composite test based on *in vitro* measurements of reactive oxygen species (ROS) and adenosine triphosphate (ATP). Drugs tested in this system were those that had been described as banned, withdrawn, unapproved or severely restricted by European and American governments. Zhang et al [17] tested the drug categories that included black box warnings for hepatotoxicity or drugs associated with acute liver failure.

O'Brien et al [18] generated a DILI classification using High Content Biology Screening (HCS) by looking at the effects of 300 drugs on various mechanisms of toxicity such as stress pathway activation, organelle function, oxidative stress, DNA Damage, Cell Cycle and cytoskeletal integrity. For the HCS experiments, HepG2 and primary rat hepatocytes cells were exposed for different time intervals representing acute, early and chronic drug level exposures with the objective of identifying toxicity biomarkers.

High-throughput screening (HTS) assays are used extensively in drug discovery as a method for identifying small molecule 'hits' against a target of interest for potential efficacy. Similarly, HTS can be used as an early screen to detect molecules with the potential to cause unacceptable toxicity. One thousand chemicals were tested in 500 different assays for the ToxCast project [19] and approximately 10,000 chemicals were tested by 50 different assays for the Tox21 project [20]. Both ToxCast and Tox21 assay data were connected with the DILI classification information in LTKB. LTKB also contains information from approximately 150 compounds tested on human hepatocytes to determine DILI potential at the National Center for Toxicological Research where several drugs causing severe DILI were identified [17].

3.5 Toxicogenomics (TGx) in LTKB for DILI prediction

TGx databases contain information regarding the effect of a drug on the toxicogenomic response of targeted organs including liver, kidney, and primary hepatocytes from both rats and humans. One of the comprehensive TGx databases is based on microarray gene expression profiling conducted in the Japan Toxicogenomics Project [21, 22] (<http://toxico.nibio.go.jp/open-tggates/search.html>.) This TG-GATEs database has been linked to DILI information in LTKB. The same approach was also implemented to link LTKB to DrugMatrix toxicogenomics data hosted by the National Toxicological Program, National

Institute of Environmental Health Sciences (<https://ntp.niehs.nih.gov/drugmatrix/index.html>). [23].

4. Utility of LTKB

The LTKB provides a centralized repository for data for the study of DILI. Particularly, the DILI classification data in LTKB could be a useful resource to connect phenotype with molecular and mechanistic data for developing biomarkers, predictive models and assessing data from emerging technologies such as high-throughput screening, high-content screening and in silico methodologies (Figure 3). For example, bioinformatics approaches [24-28] including Structure-Activity Relationship (SAR) and Quantitative Structure-Activity Relationship (QSAR) can be used to establish a connection between the structures of a chemical and its DILI risk [29]. In one study, approximately 500 drugs were analyzed using a QSAR approach [24, 30], where molecular descriptors were generated using Mold2 [28], and structure-DILI relationships were identified using the Decision Forest algorithm [27]. A second study is developing a DILI predictive toxicological approach to identify DILI-associated side effects [24]. Metabolism-related parameters that have potential to cause DILI [25, 26, 31, 32] are also of interest. Importantly, after the identification of essential parameters indicating DILI, a relationship model was established to predict DILI called “Rule of Two” [31] which flags each drug for its DILI potential based on its lipophilicity (measured by its $\log P \geq 3$) and a daily dose of ≥ 100 mg/day. More recently, DILI severity quantitative scores were developed on the basis of a drug’s lipophilicity, daily dose, and its capability to form metabolites [32].

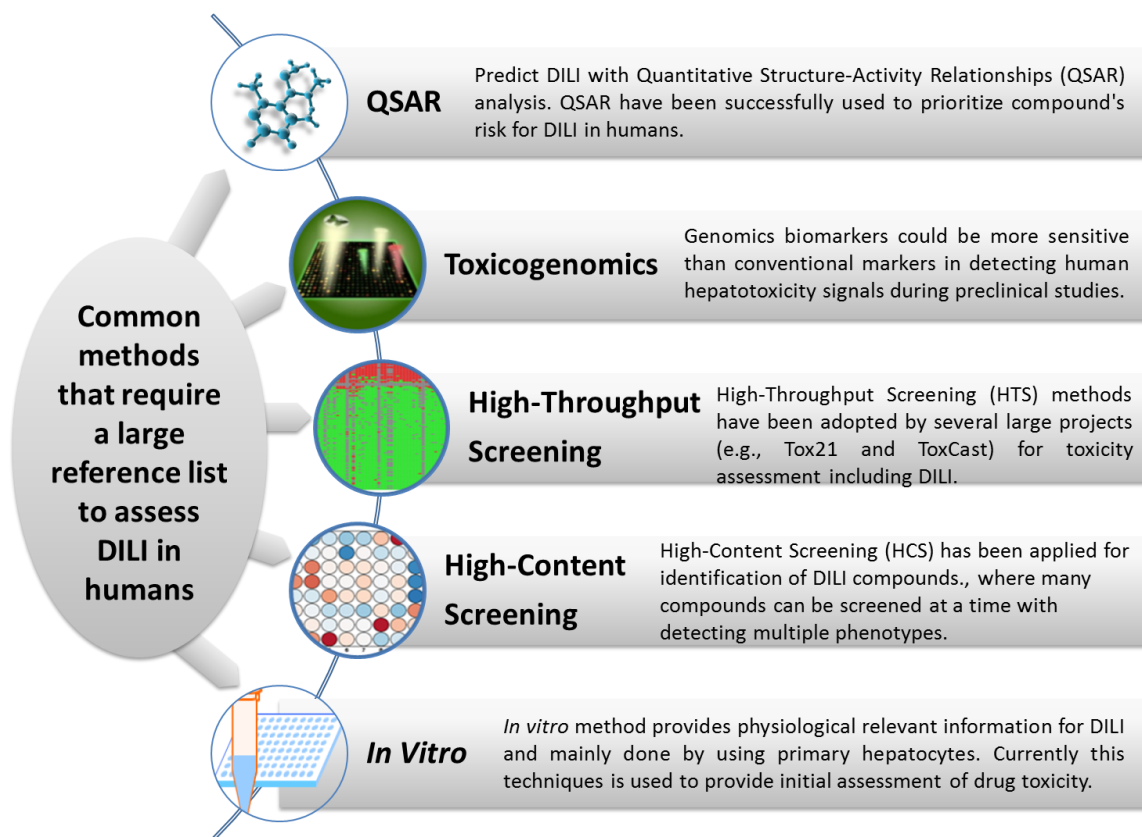


Figure3. Key methodologies that require a large *drug* reference list for assessing toxicity. Most of these methods *are* employed to screen simultaneously hundreds of drugs to *assess* DILI potential. *LTKB integrates information generated from all these methodologies into one tool that can be interrogated.*

5. Expert Commentary

Liver toxicity is a multifactorial problem and its prediction is challenging due to the diverse mechanisms involved in the causation of DILI. One of the major issues is a lack of translation of preclinical data to clinical findings; this poses challenges in interpretation of preclinical data to assess and predict human risk. This may be improved by including clinical severity information for known drugs into models that predict DILI. Another important aspect is how drugs interact with individual patients, sometimes generating idiosyncratic DILI associated with specific risk factors, often with an underlying immune-mediated mechanism. A more complete understanding of the dataset can be obtained by integration of all drug information such as chemical structures and properties with toxicogenomics information, *in vitro* and *in vivo*

information. This integration not only provides a complete preclinical picture for a particular drug, it may also help to understand the responses to a drug at various level of complexity from drug properties, genomic signals and cellular response to *in vivo* observations.

A second key issue is that it is vital to understand and incorporate more sophisticated descriptors of clinical outcome of liver injury into predictive models. Currently, most DILI studies center on a simple prediction of DILI severity but DILI has multiple complexities such as the different types of injury patterns, pathologies and associated clinical progression. It is important that we develop the ability to predict different types of DILI that may be associated with disease severity. Furthermore, it would be highly valuable to incorporate into predictions the observations that some therapeutic drug categories are more prone or predisposed to liver injury related issues, and that some drugs can cause damage to multiple organs.

6. Five Year View

The next five years will inevitably feature significant advances in computation and automation in this field. As depicted in Figure 4, much progress has been made in our ability to assess DILI severity and many drugs (>1000 drugs in LTKB) have been classified by their probability to cause DILI. With advancements in the accuracy of DILI classification, model accuracy will also increase. This knowledge will drive the enhanced prediction of not only DILI severity, but also DILI patterns and disease types. Another key development could be automatic inclusion into the database of DILI-related FDA labeling information and post-marketing data available at the FDA Adverse Events Reporting System (FAERS) to build content and enhance the DILI classification system. With the continuing growth of diverse data in LTKB, an ultimately aim would be integration of progressively diverse datasets from multiple sources and technologies for improved DILI assessment.

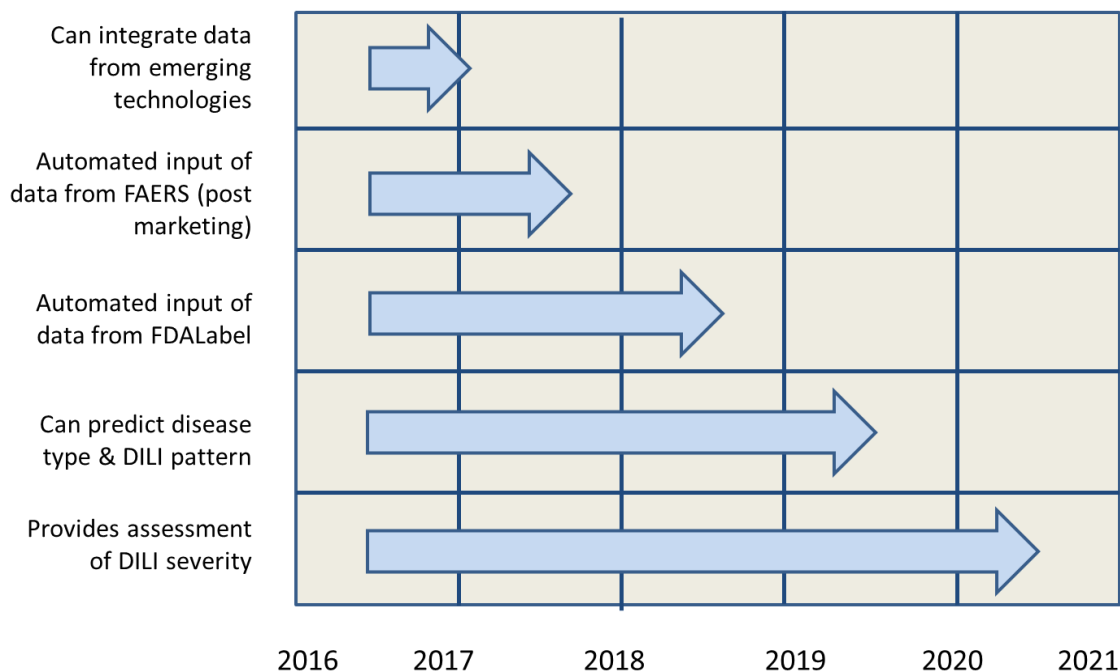


Figure 4. A schematic projection of currently established DILI related technologies and their status in the upcoming 5 years. Arrows depict estimations of the future developments for each approach from the current day to full potential.

7. Summary

With the rapid advancements in *in vitro*, *in silico*, HTS, and HCS technologies, it is becoming more important than ever to have an accurate classification of toxicological endpoints (such as DILI) covering a large number of drugs and drug classes. With this in mind, different DILI classifications can be integrated to provide a more comprehensive understanding of DILI. The scientific community is united in its desire to define the likelihood of a drug causing liver injury but different approaches have been taken to quantify DILI risk. As a result, there are many different ways of characterizing DILI and many different approaches to the development of biomarkers based both on traditional techniques and on the use of emerging technologies such as HTS, HCT and TGx. Many of these biomarkers remain to be validated, confounded by some of the inaccurate or unhelpful classifications of DILI. LTKB integrates and compares DILI terminology and classification. Highlighting the inclusion of DILI related information in LTKB provides the opportunity for a comprehensive understanding and analysis of various available DILI classifications. This is the first time that such a detailed analysis of different sources and types of DILI-related information has been presented.

While the LTKB can be effective in supporting the development of predictive models for DILI, it also provides assistance in improving our understanding of underlying mechanisms of DILI. It offers centralized information on DILI including its biological complexity and users can see 'at a glance' all the available DILI information. This is a useful resource for researchers, clinicians and regulators permitting a rapid assessment of DILI related risk but also as a prompt to consider mechanisms and severity. This knowledgebase provides opportunities for researchers building integrated models for predicting DILI, provides support for reviewers making DILI related queries, and serves clinicians as a DILI resource.

8. Key issues:

- A comprehensive drug list accurately classified for its potential to cause human liver injury is an important resource to support drug-induced liver injury (DILI) studies.
- DILI classification raises substantial challenges since the topic is controversial; different publications and databases have used different methods raising the need for harmonization and integration.
- With increasing demand for quick turn-around procedures to predict DILI, high-throughput and in silico approaches are being used in this field. Centralized DILI related information can provide a 'one-stop-shop' to address key questions like risk of DILI and its incidence and severity.

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Competing interests

Dr. Ruth Roberts is co-founder and co-director of Apconix, an integrated toxicology and ion channel company that provides expert advice on nonclinical aspects of drug discovery and drug development to academia, industry and non-for-profit organisations.