Model Developer Perspective on Contexts of Use and Their Addressability

Complex In Vitro Model (CIVM) Workshop

Presented by:
Danny Levner, PhD, Chief Technology Officer, Emulate
Patient Safety Requires Modernization

A review of 578 discontinued and withdrawn drugs in Europe and the United States showed that nearly half halted distribution due to post-approval toxicity.¹

- A 2008 study concluded that animal models predicting drug toxicity in humans may have sensitivity and specificity values below 70%².
- A 2012 analysis of 93 post-approval drugs with serious toxicity effects found that only 19% of them showed indications of toxicity in animal studies.³

► In September 2021, the European Parliament voted overwhelmingly in favor of a resolution to phase out animal use for research, testing, and education by adopting an action plan.

► On December 29, 2022, the FDA Modernization Act 2.0 was signed into law, encouraging sponsors to submit data from human-relevant technologies to support their IND submission.

► On June 13, 2023, Bill S-5 in Canada was signed into law, mandating the Ministers of Health and Environment to publish a plan within two years which promotes non-animal toxicity testing.

Liver Toxicity is a Leading Cause of Late-Stage Drug Attrition

Clinical Trial Failures¹

Post-Market Withdrawals²

Severe Market Failures

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic indication</th>
<th>Patient Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benoxaprofen</td>
<td>Rheumatoid Arthritis</td>
<td>139 Deaths</td>
</tr>
<tr>
<td>Fialuridine</td>
<td>Hepatitis B</td>
<td>5 Deaths &amp; 2 Liver Transplants</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>Clinical Depression</td>
<td>20 Deaths</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>Type II Diabetes</td>
<td>61 Deaths &amp; 7 Liver Transplants</td>
</tr>
<tr>
<td>Ticrynafen</td>
<td>Hypertension</td>
<td>25 Deaths</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>Antibiotic</td>
<td>5 Deaths</td>
</tr>
</tbody>
</table>

Patient Impact: 255 deaths 9 Liver Transplants

¹. Valentin & Redfern 2017 Toxicologist, 1722
². Siramshetty et al., 2016 Nucleic Acids Research, 44 (D1), D1080-D1086
Drug-Induced Liver Injury Remains a Patient Safety Concern

- **Phase IIb stopped**: Vupanorsen associated with dose-dependent elevations in transaminases and liver fat.
- **Phase III partial hold**: Tolebrutinib associated with DILI in patients who may be predisposed to DILI.
- **On market**: 2 patient deaths resulting from acute liver failure following Zolgensma treatment.
- **Phase III partial hold**: Evobrutinib caused liver damage in 2 patients, limiting further enrollment.
- **Phase Ib stopped**: ALG-020572 associated with elevations in transaminases and risk of DILI.
- **Phase Ib raises flag**: Lumakras in combination with Keytruda or Tecentriq resulted in 50% of patients with severe liver toxicity.
- **Phase II terminated**: KVD-824 caused liver enzyme (ALT/AST) elevations in multiple patients in all treatment groups.
- **Phase Ib paused**: SZN-1326 associated with increased transaminases in healthy participants.
THE PAST:
Static & Non-Human Biology

THE FUTURE:
Integrated Human Biology
Several Advanced Liver CIVMs on the Market

Some examples:

1. InSphero 3D InSight™ Human Liver Spheroids (Hepatocyte + Kupffer cell co-culture)
2. Emulate Liver-Chip (Quad culture)

- Top Channel
- Extracellular Matrix
- Hepatocytes
- Porous Membrane
- Stellate Cells
- Kupffer Cells
- Endothelial Cells
- Bottom Channel
Liver CIVM Toxicology Contexts of Use: Categorization

Predictive Toxicology

- Seeking to *predict* risk of human toxicity
- Compound has *not shown* prior indications of toxicity
- CIVM study may be performed *alongside*, *prior to* or *after* animal studies

Mechanistic Investigational Toxicology

- Seeking to *understand* observed toxicity
- Compound *has shown* indications of toxicity in another system
- CIVM study performed *after* animal models or human administration
CIVMs: Regulatory Purview

- **Supports 3R**: ✔
- **In regulatory purview**: ✔

### Traditional in vitro models
- **Animal dose-ranging studies**
- **Animal GLP studies**
- **CIVM**
- **Evaluate DILI risk**
- **Liver-Chip DILI score**
- **Evaluate DILI risk**
- **Regulatory submission**

### Standard weight of evidence studies
- **Animal dose-ranging studies**
- **Animal GLP studies**
- **CIVM**
- **Evaluate DILI risk**
- **Liver-Chip DILI score**
- **Evaluate DILI risk**
- **Regulatory submission**

### Evaluation Criteria
- **Unacceptable risk**: Stop
- **Acceptable risk**: ✔

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- **Animal GLP studies**: ✔
- **Liver-Chip DILI score**: ✔
- **Evaluation**: ✔
- **Regulatory submission**: ✔
Certainly an area of need, but it has two significant problems from the developer’s perspective:

<table>
<thead>
<tr>
<th>Without CIVM</th>
<th>With CIVM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species 1 Liver Finding</td>
<td>Species 2 Liver Finding</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
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</tbody>
</table>

1. Animal CIVMs are a challenge for developers because they are separate products from human version:
   - Different cell sourcing challenges
   - Different protocol
   - Different assay kits (e.g. ELISA antibodies)
   - Separate validation studies (i.e. test against animal tox)

2. What clinical data do we use for validating the human CIVM?
   - Many drugs with animal tox are left on the shelf (so no human data available)
   - Developers can’t typically access the animal findings for drugs with human data
Narrow DILI Context: Developer Perspective

• Example of narrow DILI context:
  o Prediction of immune-mediated DILI

• Development status:
  o Initial demonstrations of feasibility have been presented
  o Limited data and commercial availability of liver products that include circulating immune cells

• When would you run such an assay?
  o If you already know to suspect that specific type of DILI (or else is this broad-scope DILI prediction?)
  o But why do you already suspect the specific type of DILI?
    • Because you saw indication of it in animals? Then this is mechanistic investigational tox
    • Because this is a frequent issue for this drug class?

• As for immune-mediated DILI for biologics:
  o With so few examples, what drug set do we use for validation?
Broad DILI Context: Developer Perspective

- Broad-scope DILI prediction seems like a relevant problem:
  - DILI leads to ~13% of tox failures¹
  - DILI leads to ~21% of withdrawals²
  - 8 high-profile DILI clinical trial holds in last 18 months
  - DILI can be readily life threatening

- Development status:
  - At least three CIVM manufacturers offer commercial-grade broad-scope DILI assays
  - At least two significant studies quantified CIVM accuracy across broad sets of drugs

- Integration into pharma and regulator workflows:
  - No manufacturer is suggesting that CIVMs replace animals at this stage! Use alongside animals
  - Use of CIVMs alongside animals build animals as a safety net, and improve upon animals with human-relevant data

¹ Valentin & Redfern 2017 Toxicologist, 1722
² Siramshetty et al., 2016 Nucleic Acids Research, 44 (D1), D1080-D1086
Evaluation of the Emulate Liver-Chip following the IQ MPS Guidelines for DILI

Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology


Communications Medicine, Article number: 154 (2022) | Citeseer Article

29k Accesses | 27 Citations | 670 Altmetric | Metrics

Evaluation of the InSphero InSight™ 3D Hepatic Spheroids

Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury

William K. Preedy, Aba K. Fotedar, Jennifer Yag, Claire Hughes, Brian Baldwin, Mark S. Pilling, Andrew Shuster, John P. Nurse, Simon Maitner, Daniel Williams

Received: 4 April 2017; Accepted: 3 May 2017

Abstract Drug-induced liver injury (DILI) continues to be a major source of clinical failure, preclinical errors, drug rejections, and post-marketing withdrawal of drugs. Accordingly, there is a need for more predictive tools to assess hepatotoxicity risk in drug discovery. Three-dimensional (3D) hepatic spheroids have emerged as promising tools to assess mechanisms of hepatotoxicity, as they demonstrate enhanced liver function, metabolic activity, and ability to culture against retroviral screening for drug safety. In vitro models of drug-induced liver toxicity have been demonstrated with relative modules of hepatocellular changes. However, a comprehensive evaluation of these models is lacking. Here, we estimated the potential value of 3D human liver microtissues (HLMT) to identify liver hepatotoxicity using a panel of 10 drugs with and without clinical DILI has been assessed in comparison to human primary hepatocytes (PHH). Compounds were tested in 96-well plates at 10 concentrations and in vitro data were evaluated using indirect 4T1, relative cell count, and relative activity reporter. Utilizing hematoxylin and eosin, while specific details are consistent across all assays. In addition, HLMT is performed in PKH in correctly classifying hepatocytes from different physiological states of cells. The HLMT demonstrated sufficient capability to warrant evaluation in future drug discovery. Hematoxylin and eosin staining (with DAPI, H&E) for the cell structure. Taken together, this study represents the validation analysis of 3D hepatic spheroid cultures to new and supports their utility in hepatotoxicity risk screening in drug discovery.
Out of 27 drugs, 22 of which caused DILI in humans:

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Animal models</td>
<td>0% (reference)</td>
<td>100% (reference)</td>
</tr>
<tr>
<td>Spheroids</td>
<td>47%</td>
<td>100%</td>
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<tr>
<td>Liver-Chip</td>
<td>87%</td>
<td>100%</td>
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The Emulate human Liver-Chip correctly identified 87% of drugs that caused DILI in humans, despite passing through animal testing.

The Liver-Chip could have prevented 242 deaths in the clinic due to its predictive power.

*Value associated with 27 drugs that caused DILI despite passing animal testing evaluations. Source: Ewart et al., 2022, Communications Medicine, 2: 154
What Does 87% Sensitivity Mean?

Hepatotoxic drugs permitted into the clinic after evaluation in animals

7.5x reduction in # of drugs inducing liver injury

Into the clinic

100% 13%
Integrating Liver-Chip data into pharmaceutical decision-making processes

Daniel Levner and Lorna Ewart

Liver-Chip Score Mapped to Garside DILI Severity

<table>
<thead>
<tr>
<th>Liver-Chip DILI Score</th>
<th>Garside Score Prediction (PPV)</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>Score &lt;= 31</td>
<td>100% 0% 0% 0%</td>
<td>Almost certain to cause death or acute liver failure and be withdrawn or receive black box warning due to hepatotoxicity</td>
</tr>
<tr>
<td>31 &lt; Score &lt;= 160</td>
<td>67% 33% 0% 0%</td>
<td>Almost certain to cause high acute liver failure, but unlikely to be withdrawn or receive black box warning due to hepatotoxicity</td>
</tr>
<tr>
<td>160 &lt; Score &lt;= 240</td>
<td>0% 100% 0% 0%</td>
<td>Almost certain to cause high acute liver failure, but unlikely to be withdrawn or receive black box warning due to hepatotoxicity</td>
</tr>
<tr>
<td>240 &lt; Score &lt;= 375</td>
<td>0% 50% 50% 0%</td>
<td>Almost certain to cause symptoms likely to be withdrawn or receive black box warning due to hepatotoxicity</td>
</tr>
<tr>
<td>Score &gt;= 375</td>
<td>Predicted non-toxic with 87%</td>
<td></td>
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Liver-Chip Decision Matrix

Utilizing Human Liver-Chip and Animal in vivo findings improves preclinical decision making

Without Liver-Chip | With Liver-Chip |
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<td>-</td>
<td>+</td>
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<tr>
<td>+</td>
<td>Investigational Study</td>
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Objection: DILI prediction is not an area of significant concern outside of specific mechanisms
  - Recent failures and clinical trial holds suggest that DILI is still an area of concern for small molecule drugs across a range of mechanisms

Objection: CIVMs may miss toxic compounds that animals would have caught
  - CIVM developers have adopted the strategy of using liver CIVMs alongside animals in regulatory contexts
  - A “best of both worlds” strategy: retain the mechanistic breadth of animal models, while supplementing them with human-relevant data
  - Approach aims to permit CIVMs to “only improve” over the status quo

Objection: CIVMs will fail good drugs (false positives), doing more harm than good
  - Some liver CIVMs have shown 100% specificity (no false positives)
  - This is not a fluke: different systems are prone to different types of errors; the Liver-Chip may miss some toxic drugs, but there is no indication that it has ever “hallucinated” a nonexistent mechanism of toxicity
Concluding Remarks

In choosing contexts of use for defining liver CIVM qualification, we should keep in mind:

- Regulators can rank areas of need, and developers can rank how addressable each area is
- We need to stay in the regulatory purview
- Is data to conduct validation available?

The overlap of these factors may not be where we initially expected

- It would be less helpful to produce guidelines for contexts of use that developers can’t yet address
- It would be more effective to guide developers within contexts of use that they can address, and use the guidelines to ensure that regulator needs are met