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Lead Scientist and Manager, DILI-sim

Click to view Biosketch and Presentation Abstract
or page down to review presentation
Serum Cytokeratin-18 as a Biomarker for Liver Injury?

19 March 2015

Brett A. Howell, Ph.D.
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The DILI-sim Initiative is a partnership between the Hamner Institutes and Pharmaceutical Companies to Minimize DILI.

**Overall Goals of Simulation Project**
- Improve patient safety
- Reduce the need for animal testing
- Reduce the costs and time necessary to develop new drugs
Examples of DILIsym® Applications

**IVIVE**

**Rank compounds by risk**

**Preclinical biomarker study design**

**Preclinical**

**in vitro**

**in vivo**

**Single Ascending Dose**

**Phase II/III/IV**

**Clinical**

**DILI Dose Response Estimation**

**Clinical biomarker analysis**

**Predicting variability in response**

Institute for Drug Safety Sciences
Clinical Concern with Compound X

- A novel compound (Compound X) is in development to address an important, unmet medical need
  - Target patient population would be treated in the in-patient setting

- Clinical concern
  - Dose dependent elevations in serum ALT and other biomarkers were observed in phase I clinical studies
  - No Hy’s Law cases observed
## Compound X Serum Biomarkers

### Clinical Data

<table>
<thead>
<tr>
<th>Daily Dose Level and Infusion Length</th>
<th>ALT Elevations within Clinical Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67x - long</td>
<td>None</td>
</tr>
<tr>
<td>1x - medium</td>
<td>None</td>
</tr>
<tr>
<td>1x - long</td>
<td>None</td>
</tr>
<tr>
<td>1x - short</td>
<td>1/7 ALT &gt; 2x ULN</td>
</tr>
<tr>
<td>1.3x - long</td>
<td>4/7 &gt; 3x ULN</td>
</tr>
<tr>
<td>2x - short</td>
<td>2/6 ALT &gt; 3x ULN</td>
</tr>
<tr>
<td></td>
<td>5/6 &gt; 2x ULN</td>
</tr>
</tbody>
</table>

![Graphs showing ALT and miR-122 changes over time.](image)

**ALT (U/L)**

- 2X ULN
- 3X ULN

**Cleaved K18 (U/L)**

- 500
- 1000
- 1500
- 2000
- 2500

**Time (hours)**

- 0
- 96
- 192
- 288
- 384
- 480
- 576

**miR-122 (Fold Change)**

- 0
- 10
- 20
- 30
- 40
- 50
- 60

**Time (hours)**

- 0
- 96
- 192
- 288
- 384
- 480
- 576
Compound X Simulation Project Objectives

• Primary objectives for DILIsym® analysis:
  – Identify/substantiate potential mechanisms related to the hepatic effects
  – Optimize the dosing and monitoring protocols to achieve an adequate liver safety margin
DILIsym® Overview

• Multiple species: human, rat, mouse, and dog
  - Population variability

• The three primary acinar zones of liver represented

• Essential processes represented to multiple scales in interacting sub-models
  – Pharmacokinetics
  – Reactive oxygen species
  – Hepatocyte life cycle
  – Biomarkers
DILIsym® Optimization Process for Compound X Utilized *in vitro* and Clinical Data

### Laboratory Experiments and Data

- Define DILIsym® setup from mechanistic *in vitro* and PK data
- Key mechanisms in play for Compound X:
  - Oxidative stress
  - ET inhibition

### Modeling & Simulation

- Compare DILIsym® predictions to clinical outcomes
- Refine DILIsym® parameter values based on human studies

### Clinical Data

- Extrapolate for safety margin, protocol design, and dose selection
- Extrapolate to humans
- Potential Clinical Studies

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*Vaquero 2007*
Human SimPops™ was used for Compound X Simulations

- SimPops™ reflect inter-patient variability
- SimPops™ included mechanistic variability in:
  - ROS production and elimination processes
  - Apoptosis induction
  - Mitochondrial dysfunction pathways
- Three exposure levels per simulated human (low, medium, high)

### SimPops™ Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
</tr>
<tr>
<td>Hepatocyte sensitivity to ATP levels</td>
</tr>
<tr>
<td>Hepatocyte sensitivity to ROS</td>
</tr>
<tr>
<td>ROS buffering capacity</td>
</tr>
<tr>
<td>Basal electron transport chain flux</td>
</tr>
<tr>
<td>Respiratory reserve capacity</td>
</tr>
<tr>
<td>Caspase activation proficiency</td>
</tr>
<tr>
<td>Baseline GSH levels in hepatocytes</td>
</tr>
<tr>
<td>GSH precursor transport velocity</td>
</tr>
<tr>
<td>Hepatocyte regeneration mediator production</td>
</tr>
<tr>
<td>Maximum hepatocyte regeneration velocity</td>
</tr>
</tbody>
</table>

\[ N = 300 \times 3 = 900 \]
Simulations of Compound X Clinical Studies Suggest that DILIsym® Adequately Recapitulates the Observed Hepatic Effects

- 7 or fewer subjects treated per cohort vs. 900 simulations done within DILIsym®
- Dose response generally on target
- Apoptosis and necrosis were present in the simulations

<table>
<thead>
<tr>
<th>Daily Dose Level and Infusion Length</th>
<th>ALT Elevations within Clinical Data</th>
<th>Simulated ALT Elevations</th>
<th>Simulated Overall Minimum Percent Hepatocytes Viable Achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67x - long</td>
<td>None</td>
<td>0/900</td>
<td>93%</td>
</tr>
<tr>
<td>1x - medium</td>
<td>None</td>
<td>39/900</td>
<td>93%</td>
</tr>
<tr>
<td>1x - long</td>
<td>None</td>
<td>13/900</td>
<td>91%</td>
</tr>
<tr>
<td>1x - short</td>
<td>1/7 ALT &gt; 2x ULN</td>
<td>142/900</td>
<td>94%</td>
</tr>
<tr>
<td>1.3x - long</td>
<td>4/7 &gt; 3x ULN</td>
<td>160/900</td>
<td>92%</td>
</tr>
<tr>
<td>2x - short</td>
<td>2/6 ALT &gt; 3x ULN 5/6 &gt; 2x ULN</td>
<td>815/900</td>
<td>39%</td>
</tr>
</tbody>
</table>

ALT Elevation Color Key

- No elevations
- 1%-25% elevations
- 26%-50% elevations
- 51%-100% elevations

Fraction Viable Hepatocyte Color Key

- FVL > 0.95
- 0.9 < FVL < 0.95
- 0.8 < FVL < 0.9
- FVL < 0.80

*Simulated ALT elevation is 3x baseline or >= 90 U/L
Simulated ALT Dynamics were Similar to Clinical Data

- Simulations done in single simulated human (red line)
- Simulated peak ALT occurs at a similar Tmax to that observed in clinical study
Prospective Predictions of Medium Length Compound X Infusions at Increasing Doses Suggested a Three-fold Safety Margin

- Target dosing level was 1x, medium length infusion
- Predicted safety margin of 3x the target level
- Without ALT stopping criterion (results not shown)
  - Safety margin was reduced
- What makes simulated humans susceptible?
  - Antioxidant effectiveness, caspase activation capacity, and body weight (exposure)

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<th>Simulated ALT Elevations</th>
<th>Simulated Overall Minimum Percent Hepatocytes Viable Achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75x - medium</td>
<td>Study not conducted</td>
<td>4/900</td>
<td>91%</td>
</tr>
<tr>
<td>1x – medium</td>
<td>None observed</td>
<td>39/900</td>
<td>91%</td>
</tr>
<tr>
<td>2x – medium</td>
<td>Study not conducted</td>
<td>509/900</td>
<td>89%</td>
</tr>
<tr>
<td>3x – medium</td>
<td>Study not conducted</td>
<td>797/900</td>
<td>16%</td>
</tr>
<tr>
<td>4.5x - medium</td>
<td>Study not conducted</td>
<td>899/900</td>
<td>15%**</td>
</tr>
</tbody>
</table>

ALT Elevation Color Key
- No elevations
- 1%-25% elevations
- 26%-50% elevations
- 51%-100% elevations

Fraction Viable Hepatocyte Color Key
- FVL > 0.95
- 0.9 < FVL < 0.95
- 0.8 < FVL < 0.9
- FVL < 0.80

**Simulated death occurred

*Simulated ALT elevation is 3x baseline or >= 90 U/L

Clinical Data and Simulation Results
Prospective Predictions of Weight-Adjusted Compound X Dosing Suggested an Improved Safety Margin

- Equivalent weight-adjusted (WA) doses calculated
- Predicted safety margin of 4.5x the target level

<table>
<thead>
<tr>
<th>Daily Dose Level and Infusion Length</th>
<th>ALT Elevations within Clinical Data</th>
<th>Simulated ALT Elevations</th>
<th>Simulated Overall Minimum Percent Hepatocytes Viable Achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75x WA - medium</td>
<td>Study not conducted</td>
<td>0/900</td>
<td>92%</td>
</tr>
<tr>
<td>1x WA – medium</td>
<td>Study not conducted</td>
<td>16/900</td>
<td>91%</td>
</tr>
<tr>
<td>2x WA – medium</td>
<td>Study not conducted</td>
<td>502/900</td>
<td>90%</td>
</tr>
<tr>
<td>3x WA – medium</td>
<td>Study not conducted</td>
<td>813/900</td>
<td>83%</td>
</tr>
<tr>
<td>4.5x WA– medium</td>
<td>Study not conducted</td>
<td>900/900</td>
<td>15%**</td>
</tr>
</tbody>
</table>

**Simulated death occurred**

*Simulated ALT elevation is 3x baseline or >= 90 U/L; WA = weight-adjusted dosing

ALT Elevation Color Key
- No elevations
- 1%-25% elevations
- 26%-50% elevations
- 51%-100% elevations

Fraction Viable Hepatocyte Color Key
- FVL > 0.95
- 0.9 < FVL < 0.95
- 0.8 < FVL < 0.9
- FVL < 0.80

ALT stop criteria used
Compound X Simulation Project Objectives

• Primary objectives for DILIsym® analysis:
  – Identify/substantiate potential mechanisms related to the hepatic effects
    • In vitro data, cK18 data, and simulation results combined to point to oxidative stress as the most likely mechanism
  – Optimize the dosing and monitoring protocols to achieve an adequate liver safety margin
    • Daily monitoring of circulating liver enzyme levels and weight-adjusted dosing were identified as strategies for reducing risk
Novel Biomarker Assessments in Early Phases of Drug Development

• Cleaved K18 data supported the mechanism and mode of cell death suggested by the simulations

• Simulations pointed to greater sensitivity for cK18 than ALT

• How should cleaved K18 elevations be applied clinically?
  – Is apoptosis good or bad?
  – Stop-rule applications?
  – Clinically relevant levels of cK18?

Clinical Data and Simulation Results
DILIsym® Can Be Applied to the Rational Proposal of Cut-Off Levels for Cleaved Cytokeratin-18 (cK18)

- Simulations can be used to account for the number of dying hepatocytes for a given increase in ALT
  - e.g. 5 hepatocytes shown to the right
- An equivalent level of hepatocyte death can be simulated with apoptosis
- The resulting increase in cK18 can be noted and applied to suggest clinically relevant cut-offs

<table>
<thead>
<tr>
<th>Fold Change for ALT</th>
<th>Clinically relevant cut-offs in DILIsym®</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x</td>
<td>60 U/L</td>
</tr>
<tr>
<td>3x</td>
<td>90 U/L</td>
</tr>
<tr>
<td>10x</td>
<td>300 U/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fold Change for cK18</th>
<th>Clinically equivalent cut-offs in DILIsym®</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25x</td>
<td>175 U/L</td>
</tr>
<tr>
<td>1.5x</td>
<td>210 U/L</td>
</tr>
<tr>
<td>3.25x</td>
<td>455 U/L</td>
</tr>
</tbody>
</table>

Baseline ALT 30 U/L
2x baseline ALT 30+30 U/L
Baseline cK18 140 U/L
1.25x baseline cK18 140+35 U/L

Necrotic cell death inducing 30 U/L increase
Equivalent cell death via apoptosis induces 35 U/L increase

Clinical Data and Simulation Results
Biomarker Questions Highlighted by Compound X Simulation Project

• Should emerging biomarkers (HMGB1, cK18, K18, miR122, etc.) be assessed in the clinical trial setting?

• How should such data be interpreted when considering apoptosis and necrosis, immune activation, etc., with respect to patient safety?

• What levels of cK18 should be flagged as significant?
  – DILIsym® provides a starting place to address this question
Acknowledgements

- Drug-Induced Liver Injury (DILI) Conference XV Program Organizers
- Sponsor of Compound X simulation project
- DILI-sim members