New Liver Injury Biomarkers

Jeff Lawrence and the C-Path PSTC Hepatotoxicity Working Group

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New Hepatotoxicity Biomarkers

**Needs**

**Preclinical Needs**
- ALT changes in the absence of histopathological evidence of injury
- Absence of preclinical liver signals that do appear in clinical trials
- Poorly or unmonitorable liver changes
  - Microvesicular steatosis
  - Hepatocellular proliferation
  - *Biliary epithelial hyperplasia*
  - *BSEP inhibition*
  - Hepatic inflammation
  - *Oxidative stress*

**Clinical Needs**
- detect susceptibility to DILI prior to drug exposure
- detect susceptibility to DILI during drug exposure
- differentiate DILI from other causes of liver injury
- predict the course of DILI once it occurs
- Mild or transient aminotransferase changes

**Objectives**

1. Distinguish transient (adaptive) changes from serious liver injuries associated with progressive liver functional loss and liver failure, i.e., better Hy’s law
2. Distinguish ALT changes associated with *bona fide* liver injury from those not associated with liver injury (no histological indication of injury)
3. Improve differentiation of liver injury from injuries of other organs, e.g. muscle

*Other efforts are in blue italics*
Now that we know what we want, how do we do it?

- Applications/Claims/Context of use
- Technical Assay Validation
  - fit for purpose
- Highly annotated samples to analyze
  - Standard histopathology lexicon (Histopathology Practices Guide)
  - Samples from studies to address:
    - Liver injury with various severities
    - Chemical/mechanistic diversity in liver injury induction
    - Transient liver injury
    - Liver injury progression to liver failure
    - Recovery
    - Specificity (skeletal muscle, kidney, heart, inflammation)

- Statistical Analysis Plan
- Engagement of the Regulatory Agencies
  - FDA, EMA, PMDA
- Develop Clinical Translational Plan

Longitudinal sampling
## Current Qualification Biomarkers

<table>
<thead>
<tr>
<th>Marker</th>
<th>What will it detect?</th>
<th>Tissue Expression</th>
<th>Liver localization</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine Aminotransferase (ALT)</td>
<td>Injury</td>
<td>Liver, skeletal muscle,</td>
<td>Hepatocytes</td>
<td>Glucose- Alanine cycle</td>
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<tr>
<td>Glutamate Dehydrogenase (GLDH)</td>
<td>Injury</td>
<td>liver, kidney, muscle, intestine</td>
<td>Hepatocytes (centrilobular)</td>
<td>Amino acid oxidation, urea production</td>
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<tr>
<td>Malate Dehydrogenase (MDH)</td>
<td>Injury</td>
<td>muscle, kidney, brain, intestine</td>
<td>Hepatocytes (periportal)</td>
<td>TCA cycle</td>
</tr>
<tr>
<td>Glutathione S Transferase-α (αGST)</td>
<td>Injury</td>
<td>liver, adrenal, ovary, stomach, kidney, soleus, testes</td>
<td>Hepatocytes</td>
<td>Glutathione transferase</td>
</tr>
<tr>
<td>Purine Nucleoside Phosphorylase (PNP)</td>
<td>Injury</td>
<td>Bone marrow, intestine, spleen, liver</td>
<td>Hepatocytes, Endothelial, Kupffer</td>
<td>Purine pathway</td>
</tr>
<tr>
<td>Arginase-1 (Arg-1)</td>
<td>Injury</td>
<td>liver</td>
<td>Hepatocytes</td>
<td>Urea cycle</td>
</tr>
<tr>
<td>Paraoxonase-1 (PON1)</td>
<td>Function</td>
<td>liver, diaphragm</td>
<td>Hepatocytes</td>
<td>Esterase, protects lipoproteins from lipid peroxidation</td>
</tr>
<tr>
<td>F-Protein (HPPD)</td>
<td>Injury</td>
<td>Liver</td>
<td>Hepatocytes</td>
<td>allo-4-hydroxyphenylpyruvate dioxygenase</td>
</tr>
<tr>
<td>miR122 (maybe others)</td>
<td>Injury</td>
<td>liver</td>
<td>Hepatocytes</td>
<td>small non-coding RNAs repress translation</td>
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</table>

Red colored spaces identify diversity from ALT
Sample Inventory

We have compiled a dataset of 32 compounds in 31 studies resulting in a database of 1519 samples with biomarker measurements and corresponding histopathology

• Includes 8 novel (proprietary) compounds
• Liver pathologies include
  • Acute liver injury: multilobular, centrilobular, and periportal necrosis, biliary inflammation, hypertrophy/induction
• Kidney injury
• 8 contributing member companies
• This required extensive curation and QC of the data

New studies/designs
• Discordant ALT/histopathology studies
  • Dexamethasone, 3 proprietary compounds
• Muscle vs liver injury studies
**Statistical Analysis**

GLDH provides improved specificity without sacrificing sensitivity

__ROC Curves for Comparisons__

**ROC Curve Comparison - ALT versus GLDH**

From 35 to 18% specificity

Note the improved specificity at high sensitivity

<table>
<thead>
<tr>
<th></th>
<th>AUC (model)</th>
<th>AUC (ALT)</th>
<th>AUC diff.</th>
<th>AUC diff p-value</th>
<th>IDI</th>
<th>Relative IDI</th>
<th>IDI p-value</th>
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<tbody>
<tr>
<td>PPMG</td>
<td>0.908</td>
<td>0.870</td>
<td>0.038</td>
<td>0.001 **</td>
<td>0.126</td>
<td>35.4%</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>GLDH</td>
<td>0.903</td>
<td>0.870</td>
<td>0.033</td>
<td>0.006 **</td>
<td>0.096</td>
<td>26.9%</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>MDH</td>
<td>0.605</td>
<td>0.870</td>
<td>-0.264</td>
<td>&lt;.001 ***</td>
<td>-0.301</td>
<td>-84.7%</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>PNP</td>
<td>0.793</td>
<td>0.870</td>
<td>-0.077</td>
<td>&lt;.001 ***</td>
<td>-0.063</td>
<td>-17.7%</td>
<td>&lt;.001 ***</td>
</tr>
<tr>
<td>PON1</td>
<td>0.766</td>
<td>0.870</td>
<td>-0.104</td>
<td>&lt;.001 ***</td>
<td>-0.248</td>
<td>-69.7%</td>
<td>&lt;.001 ***</td>
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</table>
Statistical Analysis
Liver versus kidney injury

- Strong changes with liver injury
- No significant change with kidney injury
- Next assessments
  - Non-necrosis liver pathologies (hypertrophy, biliary epithelial cell hyperplasia)
  - Different forms of Discordant ALT/histopathology mechanisms
  - Muscle versus liver injury
ALT Can Increase Without Histopathological Evidence of Liver injury

- Standard GLP Non-human primate toxicity study - 3 months exposure
- Note the large increase in serum ALT
- **No histopathological evidence of liver or muscle injury**
- Discordant ALT/histopathology finding

- Additional samples assessed for alternative liver injury biomarkers including GLDH and SDH
- Note similar elevations with ALT, AST, and GLDH
- **No elevation with SDH**
  - SDH response provides POC for a new biomarker for this mechanism-how generalizable to other cmpds?
Acetaminophen Study Design
Transient injury, Injury to liver failure, recovery

**Study Design**

**Dose Levels:** mkd, po, daily dosing
- Vehicle
- 500 (transient)
- 1000 (transient, severe injury)
- 1500 (severe injury, liver failure)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Start (D1)</th>
<th>Treatment</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Day:**
  - A: 2, 4, 5, 7, 15, 21
  - B: 4, 6
  - C: 2, 4
  - D: 2
  - E: 2

**Note that almost all animals had ALT that were returning to normal with continued dosing through day 7.**

**Out of 60 animals treated at 1500 mg/kg/day, only 4 mortalities were observed- All other animals adapted.**

**Mortalities**

**Serum ALT (IU/L)**

**Dose (mg/kg) Severity**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Min</td>
<td>5/10</td>
<td>0/10</td>
<td>1/10</td>
<td>0/9</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>1000 Min</td>
<td>7/10</td>
<td>3/10</td>
<td>2/10</td>
<td>1/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>1500 Min</td>
<td>1/10</td>
<td>7/10</td>
<td>2/9</td>
<td>1/9</td>
<td>2/10</td>
<td>0/10</td>
</tr>
<tr>
<td>500 Mild</td>
<td>0/10</td>
<td>1/10</td>
<td>0/10</td>
<td>0/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 Mild</td>
<td>3/10</td>
<td>1/10</td>
<td>0/10</td>
<td>0/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1500 Mild</td>
<td>2/10</td>
<td>4/9</td>
<td>2/9</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>
Improve prediction of severe outcome (liver failure) 
i.e., better Hy’s law

- When injury persists without adequate regeneration, functional mass declines
- When functional mass declines below a threshold, bilirubin increases
- Hy’s law (Bili + ALT) is too late (mortality is too high)
- Space for improvement in shaded in red

How much liver mass is required to eliminate bilirubin?

- $100\%$
- $80\%$
- $60\%$
- $40\%$
- $20\%$ remaining liver mass
How Could We Modulate Liver Mass Via Hepatectomy?

A surgical model would:
- minimize confounding factors
- small molecule non-specificity at toxic does,
- inflammation
- provide better knowledge of the amount of liver mass that has been removed

Hepatectomy Survival

<table>
<thead>
<tr>
<th>% Hepatectomy</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>67%</td>
<td>Variable</td>
</tr>
<tr>
<td>90%</td>
<td>100% @ 7 days</td>
</tr>
<tr>
<td>95%</td>
<td>67% @ 7 days</td>
</tr>
<tr>
<td>97%</td>
<td>0% by 4 Days</td>
</tr>
</tbody>
</table>

* animals that survived to Day 4
Discovering New Liver Functional Mass Biomarkers

- Partial Hepatectomy would provide a way to remove specific amounts of the liver (functional mass)
  - However, the liver is really good at regenerating and its mass is replaced very quickly
- Can we block hepatocyte regeneration?
  - 2-AAF (Solt-Farber model)
- How much liver is needed for survival?
  - Especially if we prevent regrowth

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**Diagram:**
- 2-AAF
- Partial Hepatectomy
- Necropsy
- % remaining liver mass:
  - 80%
  - 50%
  - 33%
  - 10%
- Bleeds
- Time
Conclusions

• The PSTC efforts have established a dialog between the nonclinical scientists, industry clinicians involved in safety monitoring, and the regulatory scientists that has led to a better understanding of DILI biomarker needs.

• Through our work in the PSTC we have endeavored to build agreed upon evidentiary standards of how to assess performance of DILI biomarkers for use in specific contexts.

• Careful study designs are needed to test the context of use in question.
  • General injury study designs cannot test the performance of biomarkers to improve Hy’s law.

• Discovery of biomarkers of liver functional mass require unique study designs to isolate the functional component from the injury component.

• Improved performance of biomarkers depends not on the AUC of the ROC curve, but on where the improvement occurs.
  • This is especially important if improved specificity is the key need.

• There are also needs for more specific biomarkers to help bring novel therapies to patients faster.
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  - Nick King
  - Rich Miller

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  - Jim Reindel
  - Mark Smith

PSTC Hepatotoxicity Working Group

<table>
<thead>
<tr>
<th>Company</th>
<th>Full Name</th>
</tr>
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<tbody>
<tr>
<td>Abbott</td>
<td>David Desmond, Eric A Blomme, Jon Maher</td>
</tr>
<tr>
<td>Amgen</td>
<td>Jeff Lawrence</td>
</tr>
<tr>
<td>AstraZeneca</td>
<td>Gerry Kenna, Mark H Steinberg, Matt Jacobsen, Yvonne Dragan</td>
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<tr>
<td>Boehringer-Ingelheim</td>
<td>Jing Yuan, Ray Kemper</td>
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<tr>
<td>Bristol-Myers Squibb</td>
<td>Frederic Moulin</td>
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<tr>
<td>C-Path</td>
<td>Elizabeth Walker, Eric Thompson, Nick King, Phil Rossi</td>
</tr>
<tr>
<td>Daiichi Sankyo</td>
<td>Martins Adeyemo, Shinya Sehata</td>
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<tr>
<td>EMA</td>
<td>Sonja Beken, Markku Pasanen</td>
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<tr>
<td>FDA</td>
<td>Richard Beger, Donna Mendrick, Joseph Hanig, William Salminen</td>
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<tr>
<td>Genentech</td>
<td>Dylan Hartley, Jacqueline Tarrant</td>
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<tr>
<td>GlaxoSmithKline</td>
<td>Christine Hunt, Holly Jordan, Jodi Boysza, Lindsey Webster</td>
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<tr>
<td>Johnson &amp; Johnson</td>
<td>Ameesha Batheja, Michael McMillian, Sofie Starckx</td>
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<tr>
<td>Lilly</td>
<td>Arie Regev, Craig Thomas</td>
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<tr>
<td>Merck</td>
<td>Valerie T. Hamilton, Philip Sherratt, Wendy Bailey</td>
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<tr>
<td>Millennium</td>
<td>Colleen Synan</td>
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<tr>
<td>Mitsubishi Tanabe</td>
<td>Naoya Masutomi</td>
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<tr>
<td>Novartis</td>
<td>Armin Wolf, Nandita Shangari</td>
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<td>Pfizer</td>
<td>Denise Robinson-Gravatt, James Mayne, Shashi Ramaiah, Shelli</td>
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<td>Schomaker, Michael Leach, David Raunig, Dean Li</td>
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<td>sanofi-aventis</td>
<td>Adrian Fretland, Christoph Funk, Heather Workman, Kyle Kolaja</td>
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<td>Douglas Keller, Zaid Jayyosi, Valerie Barlow</td>
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