Qualification of clinical biomarkers of DILI: Current gaps, research efforts, and future directions

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Drug-induced liver injury (DILI)
Major reason for regulatory action since decades

Withdrawals

Reasons for withdrawals

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Drug related liver injury

Key challenges

• Drug induced liver injury is the leading cause of acute liver failure in the United States, accounting for about half of all cases

• In the post-approval setting, DILI is a leading cause of regulatory actions, including drug withdrawals, label changes and boxed warnings

• Across the industry, we regularly loose promising candidates due to DILI
  – A part of those may be false positives

• Of predominant concern are idiosyncratic, hepatocellular types of DILI
  – Non-dose dependent (?)
  – Rare
  – Not predictable (as yet)
  – High rate of liver failure, often fatal outcome

• A major issue is the lack of suitable markers allowing for
  – Early signal detection
  – Mechanistic assessment
  – Robust prediction of clinically relevant effects
  – Risk assessment in individual patients
Standard liver tests: ALT, AST, AP, γGT, bilirubin

Some shortcomings

• Lack of both sensitivity and specificity in terms of indicating causal relationship of liver injury to drug exposure
• Tissue specificity suboptimal
• Do not allow for differentiation between injury, upregulation, reduced clearance.
• Half life of transaminases too long to allow for close monitoring and assessment of rapid changes in liver status.
• Aminotransferase activities frequently confounded by e.g. effect of different diets and different levels of physical exercise.
• Focusing on liver only, not taking into account the involvement of the immune system.

Clear need for alternative biomarkers of drug related liver injury.
Biomarker attributes of interest

• Patient level
  – Lower injury threshold
  – Earlier time to onset
  – Larger extent of changes
  – Improved specificity
  – Better suited to monitor and predict clinical course
  – Better suited to assess causality

• Population level
  – Earlier and more specific signal detection in clinical development programs
  – Improved mechanistic insight
  – Superior in terms of identifying underlying pathology
  – Better suited to predict human risk from animal toxicity
Identifying advanced liver safety biomarkers

Key challenges for biomarker qualification

- Substantial background variability in initial candidate markers
- Biomarker response varies across different populations
- Large initial number of biomarker candidates requires substantial sample volumes to be taken
- Key target events, i.e. cases of confirmed acute DILI, suitable and accessible for qualification, are overall very rare

- Large sample sizes are required
- Multitude of patient populations need to be included

Qualification cannot be achieved by one company alone
IMI SAFE-T Consortium

Objectives

• To evaluate utility of safety biomarkers for detecting, assessing, and monitoring drug induced kidney, liver, and vascular injury in humans

• To develop assays and devices for clinical application of safety biomarkers

• To compile enough evidence to qualify safety biomarkers for regulatory decision making in clinical drug development and in a translational context

• To gain evidence for how safety biomarkers may also be used in the diagnosis of diseases and in clinical practice
SAFE-T participants
SAFE-T biobank: up and running

Clinical partners

- Patient / sample codes
- Guidelines for sample collection and processing, shipment instructions

Regulatory requirements group

- SOPs, informed consent
- Approval of sample release
- Patient – sample information

Data analysis group

- Sample approval team

Biomarker assay group

- Sample request

Samples

- 29 studies planned
- 10 studies, >60,000 aliquots
- 1538 aliquots

29 studies planned

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SAFE-T database: up and running
<table>
<thead>
<tr>
<th>Candidate biomarker</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA 122</td>
<td>Ready for sample screening</td>
</tr>
<tr>
<td>albumin mRNA</td>
<td>Ready for small sample sizes</td>
</tr>
<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
<td>Optimization phase</td>
</tr>
<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
<td>In development</td>
</tr>
<tr>
<td>Conjugated/unconjugated bile acids</td>
<td>Development necessary</td>
</tr>
<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
<td></td>
</tr>
<tr>
<td>ALT 1 &amp; 2, isoform specific</td>
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<tr>
<td>F-protein (HPPD)</td>
<td></td>
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<tr>
<td>Arginase 1</td>
<td></td>
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<tr>
<td>Keratin 18 (caspase cleaved &amp; intact)</td>
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<tr>
<td>Alpha fetoprotein (AFP)</td>
<td></td>
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<tr>
<td>Regucalcin (RGN)</td>
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<tr>
<td>Glutathione S-Transferase (GST-alpha)</td>
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<tr>
<td>ST6gal I</td>
<td></td>
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<tr>
<td>Osteopontin</td>
<td></td>
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<tr>
<td>Colony stimulating factor receptor (CSF1R)</td>
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<tr>
<td>Paraoxonase 1 (PON1)</td>
<td></td>
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<tr>
<td>Prothrombin</td>
<td></td>
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<tr>
<td>LECT2</td>
<td></td>
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<tr>
<td>Glutamate dehydrogenase (GLUD, GLDH)</td>
<td></td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase (PNP)</td>
<td></td>
</tr>
<tr>
<td>Malate dehydrogenase (MDH)</td>
<td></td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
<td></td>
</tr>
<tr>
<td>ALT1/2, isoform specific</td>
<td></td>
</tr>
</tbody>
</table>
SAFE-T Biomarker qualification process

*Elements and process flow*

**DILI BM step 1 list**
- Literature
- Databases
- SAFE-T sources

**Select**

**DILI BM step 2 list**
- Healthy volunteers
- Patients non-liver disease
- Patients liver disease
- Patients hepatotoxic drugs

**Samples**

**DILI BM step 3 list**
- Assay / stat analysis / select BMs
- DILI BM step 3 list
- DILI BM step 4 list

**Exploratory phase**
- Regulatory advice
- Assay availability / development
- Background variability
- Thresholds

**Status Q1 2012**

**Confirmatory phase**
- Regulatory advice
- Assay / stat analysis / select BMs

**Qualification**
- Assay / stat analysis / select BMs
- DILI BM final list

**Submit to health authorities**

**Regulatory approval**

**Regulatory advice**

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Ongoing prospective DILI studies

• Multi-center study in patients with suspected drug-induced liver injury
• Single-center study in rheumatoid arthritis patients
• Single-center study in patients with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) during anti-proliferative treatment
• Multi-center study in patients receiving oxaliplatin based chemotherapy
• Single-center study in colo-rectal cancer patients with liver metastases
• Multi-center study in patients with chronic hepatitis C after liver transplantation
• Multi-center study in patients on antituberculosis treatment
Ongoing prospective trials
Recruitment rates from two key studies (APHP Paris)

Acute DILI (pilot study, one of four centers)

RA patients (one center)
HMGB1 and Cytokeratin 18
Mechanism based biomarkers

Necrosis and Inflammation:
- **HMGB1** – chromatin binding protein
- Passive released by necrotic cells
- Active released by activated immune cells (hyper-acetylated (Lys NLS))
- Cytokine activity (TLR/RAGE)

Apoptosis:
- **Keratin-18** – intermediate filament protein / structural integrity
- Is cleared by caspases
- Fragment released into blood
- Full length K18 passively released during necrosis

Antoine DJ et al., 2010 Mol Med
Antoine DJ et al., 2009 Toxicol Sci

Slide courtesy Neil French, MRC CDSS
High-Mobility Group Box-1 Protein and Keratin-18, Circulating Serum Proteins Informative of Acetaminophen-Induced Necrosis and Apoptosis In Vivo

Daniel J. Antoine,‡,† Dominic P. Williams,* Anja Kipar,‡ Rosalind E. Jenkins,* Sophie L. Regan,* Jean G. Sathish,* Neil R. Kitteringham,* and B. Kevin Park* 

Mice injected with 530 mg/kg of acetaminophen
CK18 and HMGB1 show better correlation to histopathology than ALT
Patients post acetaminophen overdose (1)

Association of liver biomarkers with outcome measures

Based on Antoine DJ et al., 2012 J Hepat

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Patients post acetaminophen overdose (2)
Markers for inflammation, necrosis, and apoptosis

Association with King's College Criteria for prognosis of acute liver failure

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Patients post acetaminophen overdose (3)

**HMGB1 as a potential prognostic marker**

Acetylated HMGB1 (ng/ml)

- **Spontaneous survivors**
- **Died / Required Liver transplant**

ROC curve – survival vs Death / Liver transplant

Acetylated HMGB1

ALT

Antoine DJ et al., 2012 J Hepat
HMGB1 and CK18

Summary

• Acetylated HMGB1 may be a useful prognostic DILI marker, indicating extent of inflammation

• Caspase cleaved cytokeratin 18 may have value as a prognostic DILI marker, indicating involvement of apoptosis as protective mechanism
Human ALT1/2 isoforms

• ALT1/2 isoenzymes:
  – ALT1 is highly expressed in human liver, kidney and skeletal muscle
  – ALT2 is expressed in skeletal & heart muscle, pancreas, adrenal gland and smooth muscle in multiple organs
  – ALT assay developed at AZ measures human ALT isoforms (ALT1 & ALT2).
    ⇒ Measurement of total ALT activity, immunprecipitation of ALT1 and remeasure reminder, i.e. ALT2 activity.

• Liver surgery study:
  – Twelve patients (8 m, 4 f) undergoing open liver resection
  – Mean age 66.6 (SD ±11.6), treated for either hepatocellular carcinoma (n=1), metastases of colorectal cancer (n=7), renal cell carcinoma (n=1), malignant melanoma (n=1) or for tumors of uncertain origin (n=2)
  – Pre-operative BMI 18.4 to 31.0

• Extreme Adventure race study
  – 39 participants (31 m, 8 f), well trained, experience with Adventure races longer than 24 hours
  – Age 20 to 40 years
  – Mixed ultra-endurance exercise of running, trekking, kayaking, cycling and climbing
  – Blood samples taken before and within 20 min after the end of the race


Courtesy Björn Glinghammar, AZ
ALT1/ALT2 isoenzymes and GLDH (1)

Pre and post liver surgery and physical exercise

Average enzyme activities +/- SD, percent ALT1/2 of total ALT activity

Here is a table showing the average enzyme activities for liver surgery and physical exercise, expressed as a percentage of total ALT activity:

<table>
<thead>
<tr>
<th>Liver Surgery</th>
<th>Physical exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>GLDH</td>
</tr>
<tr>
<td>ALT1 (U/l)</td>
<td>GLDH</td>
</tr>
<tr>
<td>ALT2 (U/l)</td>
<td>GLDH</td>
</tr>
<tr>
<td>ALT1 (%)</td>
<td>GLDH</td>
</tr>
<tr>
<td>ALT2 (%)</td>
<td>GLDH</td>
</tr>
</tbody>
</table>

As can be seen from the chart, the enzyme activities show significant changes before and after liver surgery and physical exercise. The figures are courtesy of Björn Glinghammar, AZ.
ALT1/ALT2 isoenzymes and GLDH (2)

Pre and post liver surgery and physical exercise

Fold changes

![Graph showing fold changes for ALT, ALT1, ALT2, and GLDH activities before and after liver surgery or exercise.](image)

Exercise
Liver surgery

Enzyme

Activity [U/L]

ALT (U/L)
ALT1 (U/L)
ALT2 (U/L)
GLDH

4.0
3.7
5.0
1.0

11.5
11.9
6.5
22.0

Courtesy Björn Glinghammar, AZ
ALT1/ALT2 activity assays

Conclusions

• ALT in plasma increases during liver injury and skeletal muscle injury, while GLDH only increases during liver injury

• %ALT1 of total ALT increases during liver injury and decreases during skeletal muscle injury

• %ALT2 of total ALT decreases during liver injury and increases during skeletal muscle injury

• Changes are in line with the relative content of ALT1 and ALT2 in liver and skeletal muscle

• For liver injury: ALT1 explains most of total ALT changes (r=1.0, p<0.001)

• For skeletal muscle injury: ALT2 increases more than ALT1, but the increase is similar to AST (5 fold) and much less sensitive than CK (30 fold)
ALT1/ALT2 isoenzymes

Summary

• Measurement of ALT isoenzymes may not add significant information to measurement of total ALT

• ALT1/2 have been taken of SAFE-T’s priority list for biomarker qualification

• In terms of differentiating muscular from liver injury GLDH seems to be superior to ALT1/2 isoenzymes
Blood-based microRNA biomarkers for DILI
Evidence from preclinical models

miR-122
- Liver tissue specific
- Translatable to human
- Earlier detection than ALT; greater sensitivity; less variability...

Yi Zhang et al, Clin Chemistry, 2010
Wang et al, PNAS, 2009
Serum microRNAs as human DILI biomarkers
Specificity of miR-122 for liver injury

Starkey-Lewis et al., (2011)
Hepatology 54:1767

ALT miR-122 miR-192 miR-1 miR-218

Healthy Controls APAP NO ALI CKD
NON-APAP ALI APAP ALI

ALT: alanine aminotransferase
miR-122, miR-192, miR-1, miR-218

ALI: acute liver injury
CKD: chronic kidney disease
APAP: acetaminophen
non-APAP ALI:
- autoimmune
- HBV
- HCV
- Clarithromycin DILI

Starkey-Lewis et al., (2011)
Hepatology 54:1767

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Parallel to qualification: DILI biomarker discovery

Why?

• Biomarker candidates do not cover all objectives of SAFE-T DILI WP
  – Lack of susceptibility markers
  – Lack of sensitive functional markers, some pathologies poorly represented
  – Most markers identified in pre-clinical models

How?

• Based on human DILI cases from SAFE-T clinical studies
  – Acetaminophen challenge study and study in patients with acetaminophen overdose, prospective DILI study, DILI in tuberculosis patients study, HCV study, Metastatic colon cancer (oxaliplatin) study with biopsies, Liver fibrosis study

• Characteristic changes in serum proteome and metabolome expected
  – Mass spec and protein antibody array analyses of plasma samples planned

• Genetic analysis not planned, but possible collaboration with iDILIC
Plan for next three years

• Expand number of DILI centers for key prospective studies
• Reprioritise DILI biomarker candidates based on stage gate cohort data (Q1 2012)
• Incorporate new markers from biomarker discovery process into qualification process
• Complete exploratory qualification phase by Q3 2012
• Confirmatory qualification studies to be completed within two to three years time
• Explore options for collaboration with other consortia beyond PSTC
Conclusions

• SAFE-T’s systems and processes for sample collection, processing, storage, shipment, and analysis have been set up and are running well

• Data capture, storage, management, and analysis tools are in place

• Clinical studies have been initiated, but need to increase recruitment

• Data on HMGB1, cytokeratin 18, circulating microRNAs, ALT isoenzymes, GLDH, αGST, miR122, PNP, PON1 and MDH provided by consortium partners offer first insights into potential predictive and diagnostic value

• Initiated regulatory interactions via briefing meetings with EMA/FDA

• Established collaboration with Predictive Safety Testing Consortium (PSTC)

• Due to delay in start of clinical studies, SAFE-T will need extension by one year
Acknowledgements

SAFE-T consortium, in particular DILI work package

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