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

• Withdrawals due to DILI
• Non-DILI 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

Academia
- AstraZeneca
- Novartis
- Pfizer
- Lilly
- Bayer HealthCare
- Bayer Schering Pharma
- Boehringer Ingelheim
- GlaxoSmithKline
- Roche
- Academic Institutions

SMEs
- Firalis
- Interface Europe
- Argutus Medical
- EDI
- Expertise & Diagnostische Immunologie

Advisors
- European Medicines Agency
- FDA

Collaborators
- Critical Path Institute
SAFE-T biobank: up and running

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

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

- **Regulatory requirements group**
  - SOPs, informed consent

- **Data analysis group**
  - Patient – sample information

- **Sample approval team**
  - Approval of sample release

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SAFE-T database: up and running

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## DILI biomarkers – status of assay development

<table>
<thead>
<tr>
<th>Candidate biomarker</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td><strong>RNA</strong></td>
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<tr>
<td>miRNA 122</td>
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<tr>
<td>albumin mRNA</td>
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<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
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<tr>
<td><strong>LCMS</strong></td>
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<tr>
<td>High mobility group box 1 (acylated vs. non-acylated)</td>
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<tr>
<td>Conjugated/unconjugated bile acids</td>
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<tr>
<td>High mobility group box 1 (acylated vs. non-acylated)</td>
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<tr>
<td>ALT 1 &amp; 2, isoform specific</td>
<td>X</td>
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<tr>
<td>F-protein (HPPD)</td>
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<tr>
<td>Arginase 1</td>
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<tr>
<td>Keratin 18 (caspase cleaved &amp; intact)</td>
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<tr>
<td>Alpha fetoprotein (AFP)</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>
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<tr>
<td>Osteopontin</td>
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<tr>
<td>Colony stimulating factor receptor (CSF1R)</td>
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<tr>
<td>Paraoxonase 1 (PON1)</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>X</td>
</tr>
<tr>
<td>Malate dehydrogenase (MDH)</td>
<td>X</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
<td></td>
</tr>
<tr>
<td>ALT1/2, isoform specific</td>
<td>X</td>
</tr>
</tbody>
</table>

**Status**
- Ready for sample screening
- Ready for small sample sizes
- Optimization phase
- In development
- Development necessary
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)
**Apoptosis:**
- **Keratin-18** – intermediate filament protein / structural integrity
  - Is cleared by caspases
  - Fragment released into blood
  - Full length K18 passively released during necrosis

**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)

Slide courtesy Neil French, MRC CDSS

Antoine DJ et al., 2010 Mol Med
Antoine DJ et al., 2009 Toxicol Sci
Cytokeratin 18 and HMGB-1

Mouse data

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)

Assessment of liver biomarkers with outcome measures

<table>
<thead>
<tr>
<th>KCCint</th>
<th>Outcome (S or DLT)</th>
<th>Max Hepatic Enceph</th>
<th>ALT (UI)</th>
<th>Acetylated HMGB1 (ng/ml)</th>
<th>total HMGB1 (ng/ml)</th>
<th>Necrosis K18 (UI)</th>
<th>% apoptosis (based on K18)</th>
</tr>
</thead>
</table>

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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![Box plots showing the distribution of biomarker levels](image)

**AcethMGB1**
- N: 47
- Y: 31
- Alpha level = 0.05
- Root MISE = 0.4...
- log10(Ch) = 2.17

**NecrK18**
- N: 47
- Y: 31
- Alpha level = 0.05
- Root MISE = 0.5...
- log10(Ch) = 2.17

**%Apoptosis**
- N: 47
- Y: 31
- Alpha level = 0.05
- Root MISE = 0.4...
- log10(Ch) = 2.17

**ALT (U/l)**
- N: 47
- Y: 31
- Alpha level = 0.05
- Root MISE = 0.4...
- log10(Ch) = 2.17

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Based on Antoine DJ et al., 2012 J Hepat
Patients post acetaminophen overdose (3)
HMGB1 as a potential prognostic marker

Acetylated HMGB1 (ng/ml)

Died / Required Liver transplant
Spontaneous survivors

ROC curve – survival vs Death / Liver transplant

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
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

<table>
<thead>
<tr>
<th>Liver Surgery</th>
<th>Physical exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALT (UI/l)</strong></td>
<td>357</td>
</tr>
<tr>
<td><strong>ALT1 (UI/l)</strong></td>
<td>29</td>
</tr>
<tr>
<td><strong>ALT2 (UI/l)</strong></td>
<td>-13</td>
</tr>
<tr>
<td><strong>ALT1 (%)</strong></td>
<td>97</td>
</tr>
<tr>
<td><strong>ALT2 (%)</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>GLDH</strong></td>
<td>154</td>
</tr>
</tbody>
</table>

Courtesy Björn Glinghammar, AZ
ALT1/ALT2 isoenzymes and GLDH (2)

Pre and post liver surgery and physical exercise

Fold changes

![Bar chart showing fold changes in enzyme activities for different conditions.](image)

Copyright 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)
• 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

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

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

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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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Shelli Schomaker
Denise Robinson Gravatt