New liver biomarkers under study in Europe: Progress of the IMI SAFE-T* Consortium

*Safer And Faster Evidence-based Translation

http://www.imi-safe-t.eu

Drug-Induced Liver Injury Conference, March 14 - 15, 2012
Silver Spring, MD

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Outline

• IMI SAFE-T Consortium: brief overview
• Progress in 2011
• Clinical biomarker qualification program
• DILI biomarker candidates
• First experimental results
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

SMEs

Advisors

Collaborators

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SAFE-T Biomarker qualification process

Elements and process flow

Literature
Databases
SAFE-T sources

Select

DILI BM step 1 list
Evaluation
DILI BM step 2 list

Filter

Healthy volunteers
Patients non-liver disease
Patients liver disease
Patients hepatotoxic drugs

Select

Samples

Regulatory advice
Assay availability / development
DILI BM step 3 list

DILI BM step 4 list
Assay / stat analysis / select BMs

Assay / stat analysis / select BMs

Background variability
Thresholds

Regulatory advice
Assay / stat analysis / select BMs

DILI BM final list

Submit to health authorities

Regulatory approval

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Key achievements at Year 2

- Biomarker candidates prioritised, assay development well advanced
- Central biobank for sample storage up and running
- Database and data capture system up and running
- Academic sites: 12 prospective clinical studies initiated
- EFPIA partners:
  - Completed SAFE-T studies: 1
  - Retrospective samples: >6500 patients from 4 studies
  - Ongoing add-on sampling: 3 studies
  - Submitted or under preparation: 5 studies
- Initiated regulatory interactions via briefing meetings with EMA/FDA
- Established collaboration with Predictive Safety Testing Consortium (PSTC)
• New partners included in 2010/2011 to increase capacity for DIKI and DILI studies:
  – University Hospital Aachen
  – University Hospital Leipzig
  – University of Liverpool
  – University of Malaga
  – University College Dublin

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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
- Patient – sample information

Data analysis group
- Approval of sample release

Sample approval team
- Sample request

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

Biomarker assay group
- Sample request

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SAFE-T database: up and running
Ongoing prospective 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
### DILI biomarkers – status of assay development

<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><strong>High mobility group box 1 (acetylated vs. non-acetylated)</strong></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>
<td></td>
</tr>
<tr>
<td>F-protein (HPPD)</td>
<td></td>
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<tr>
<td>Arginase 1</td>
<td></td>
</tr>
<tr>
<td>Keratin 18 (caspase cleaved &amp; intact)</td>
<td></td>
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<tr>
<td>Alpha fetoprotein (AFP)</td>
<td></td>
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<tr>
<td>Regucalcin (RGN)</td>
<td></td>
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<tr>
<td>Glutathione S-Transferase (GST-alpha)</td>
<td></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><strong>Glutamate dehydrogenase (GLUD, GLDH)</strong></td>
<td></td>
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<tr>
<td>Purine nucleoside phosphorylase (PNP)</td>
<td></td>
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<tr>
<td>Malate dehydrogenase (MDH)</td>
<td></td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
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<tr>
<td>ALT1/2, isoform specific</td>
<td></td>
</tr>
</tbody>
</table>

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HMGB1 and Cytokeratin 18
Mechanism based biomarkers

**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)

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

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Markers for inflammation, necrosis, and apoptosis

Patients post acetaminophen overdose

Acetylated HMGB1 may be a 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

Based on Antoine DJ et al., 2012 J Hepat
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, immunoprecipititation of ALT1, remeasure reminder

• Liver surgery study:
  – Twelve patients (8 m, 4 f) undergoing open liver resection
  – Mean age 66.6 (SD ±11.6); 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)

• Extreme Adventure race study
  – 39 participants (31 m, 8 f), well trained
  – 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

Pre and post liver surgery and physical exercise

Fold changes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity [U/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>4.0</td>
</tr>
<tr>
<td>ALT1 (U/L)</td>
<td>3.7</td>
</tr>
<tr>
<td>ALT2 (U/L)</td>
<td>5.0</td>
</tr>
<tr>
<td>GLDH</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Exercise
Liver surgery

22.0

Courtesy Björn Glinghammar, AZ
• ALT in plasma increases during liver injury and skeletal muscle injury, while GLDH only increases during liver injury

• Liver injury: ALT1 explains most of total ALT changes

• 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)

⇒ Overall, 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
Diagnostic value of αGST

• Glutathione S transferase α (αGST):
  – Inducible phase II detoxification enzyme
  – Four isozymes of GST expressed in human and other mammals; αGST is a liver specific dimer expressed in human hepatocyte cytosol
  – High concentration in centrilobular cells: may be more sensitive than ALT and AST
  – Half life ~1 h in humans: may be useful for close monitoring
  – Low molecular weight: release into plasma may occur earlier than for ALT and AST

• Meta-analysis of four Novartis phase 1 studies using αGST for liver monitoring
  – 150 healthy subjects (108 m, 42 f), age 18 - 60, BMI 18 -32, duration 1-4 weeks
  – Key objectives:
    o Analyse correlation of αGST levels with age, BMI, and aminotransferases at baseline, and with aminotransferases during treatment (active drug and placebo)
    o Characterize time profiles of αGST as compared to ALT and AST
    o Explore to which extent αGST levels may be able to support causality assessment in case of elevated aminotransferases.
Diagnostic value of Glutathione S transferase $\alpha$ ($\alpha$GST)

Preliminary results from Novartis meta-analysis

- Earlier onset and faster resolution?
- Helpful for causality assessment in a subset of cases?

- Time to onset of enzyme elevations may be marginally shorter with $\alpha$GST
- In some patients, $\alpha$GST returns to baseline faster, possibly supporting causality assessment
Improving liver test monitoring
A glance into the future?


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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
• Inclusion of five additional academic partners has significantly increased capacity for qualification efforts
• Data on HMGB1, cytokeratin 18, circulating microRNAs, ALT isoenzymes, GLDH, αGST provided by consortium partners offer first insights into potential predictive and diagnostic value
• Collaboration with PSTC is working well
• Due to delay in start of clinical studies, SAFE-T will need extension by one year