New biomarkers for drug-induced liver injury: First insights from clinical qualification

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Michael Merz, Novartis Institutes for BioMedical Research
Outline

- DILI background
- Shortcomings of current liver safety biomarkers, requirements for advanced markers
- Biomarker qualification in the IMI SAFE-T consortium
- Initial findings for new liver safety biomarkers
- Biomarker discovery
Drug safety: room for improvement

Attrition in drug development

- Around 90% of compounds entering clinical development fail

- 30% of these failures are due to clinical safety and toxicology

Kola et al. (2004), Nat Rev Drug Discovery; 3: 711-15
• For decades, drug-induced liver injury has been one of the key safety issues in drug development and post marketing

Do we have to live with losing e.g. 1/3 of drugs in phase III and 1/6 during submission?
Withdrawals due to drug-induced liver injury (DILI)

Reducing treatment options for key disease areas

Withdrawals

- Iproniazid (1959)
- Thalidomide (1962)
- Oxyphenisatin (1967)
- Ibufenac (1970)
- Benoxaprofen (1982)
- Ticrynafen (1985)
- Methaqualon (1984)
- Perhexiline (1985)
- Alpidem (1996)
- Fenfluramine (1997)
- Tolcapone (1998)
- Tolrestat (1998)
- Triazolam (1991)
- Cisapride (2000)
- Alosetron (2000)
- Cisapride (2000)
- Ximelagatran (2003)
- Rofecoxib (2003)
- Pemoline (2005)
- Lumiracoxib (2006)
- Ximelagatran (2007)
- Troglitazone (2001)

Withdrawals due to drug-induced liver injury (DILI): reducing treatment options for key disease areas.

New biomarkers for drug-induced liver injury - M Merz - April 11, 2013
Drug-induced liver injury (DILI)

Key challenges

- DILI is the leading cause of acute liver failure in the United States
- 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 lose 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 (?)
  - Not predictable (as yet)
  - High rate of liver failure, often fatal outcome
  - Rare
- 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

- Inadequate sensitivity and specificity
- Limited predictive value, both from a translational and clinical outcome perspective
- Do not allow for differentiation between injury, upregulation, reduced clearance
- Half life of aminotransferases 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
- Not supporting mechanistic understanding
- Focusing on liver only, not taking into account immune system involvement

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

- **Patient level**
  - Lower injury threshold
  - Earlier time to onset
  - Larger extent of changes
  - Improved liver specificity
  - Better suited to monitor and predict clinical outcome
  - Better suited to assess causality

- **Population level**
  - Earlier and more specific liver 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
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
- Cases with key target response, i.e. 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 Biomarker qualification process

Elements and process flow

DILI BM step 1 list
Submit to health authorities
Literature
SAFE-T sources
Databases
Evaluation

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

DILI BM step 3 list
Samples
Assay / stat analysis / select BMs
Regulatory advice
Assay availability / development
DILI BM step 4 list
Background variability
Assay / stat analysis / select BMs
Thresholds
DILI BM final list
Qualification
Assay / stat analysis / select BMs
Regulatory advice

DILI BM step 4 list
Assay availability / development
DILI BM step 3 list
Assay / stat analysis / select BMs
DILI BM step 2 list
Evaluation
DILI BM step 1 list
Submit to health authorities

Q2 2009
Q1 2010
Q2 2011
Q2 2014
DILI biomarker selection process

Two (& a half) stage approach

Stage gate analysis

Exploratory phase

- Background variability
  - High Drop
  - Low

- Response to DILI
  - Good
  - Bad Drop

- Response to non-DILI liver disease
  - Yes Drop
  - No

Confirmatory phase

- Information on...
  - Pathology?
  - Mechanism?
  - Disease severity?
  - Drug-relatedness?
  - Clinical outcome?

Number of markers
Ongoing prospective clinical studies

*Populations and (some) key objectives*

- Multi-center international study in patients with suspected drug-induced liver injury
  - Sensitivity, specificity, predictive value (outcome), association with standard markers, time profiles

- Single-center study in rheumatoid arthritis patients
  - Specificity, association with standard markers

- Single-center study in patients with acute or myeloid lymphoblastic leukemia on chemotherapy
  - Sensitivity, specificity, predictive value (outcome), association with standard markers, time profiles

- Multi-center study in patients after liver transplantation
  - Link to histopathology, association with fibrosis progression

- Multi-center study in patients on antituberculosis treatment
  - Differentiation of susceptible patients from adaptors and tolerators

- Multi-center Swiss study in patients with suspected drug-induced liver injury
  - Specificity, predictive value, association with genetic susceptibility markers

- Single-center study in nevirapine-treated HIV patients
  - Differentiation of susceptible patients from adaptors and tolerators

- Single-center study in acetaminophen-overdose patients
  - Predictive value (outcome), association with standard markers, time profiles, mechanistic understanding
### Biomarker candidates

**Initial selection**

<table>
<thead>
<tr>
<th>Candidate markers</th>
<th>Liver specificity</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>microRNA 122</td>
<td>Highly specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>albumin mRNA</td>
<td>Highly specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
<td>Highly specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>High mobility group (HMGB-1), hypo- and hyperacetylated forms</td>
<td>Not specific</td>
<td>Hepatocellular injury, inflammation</td>
</tr>
<tr>
<td>Cytokeratin 18, full length and caspase cleaved fragment</td>
<td>Not specific</td>
<td>Hepatocellular injury, necrosis vs apoptosis</td>
</tr>
<tr>
<td>Conj./unconj. bile acids</td>
<td>Highly specific</td>
<td>Cholestatic injury</td>
</tr>
<tr>
<td>Urocanic Acid</td>
<td>Not specific</td>
<td>Cholestatic injury, biliary hyperplasia</td>
</tr>
<tr>
<td>ALT1 &amp; 2</td>
<td>Highly (ALT1)</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (GLDH)</td>
<td>Specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase (PNP)</td>
<td>Specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Malate dehydrogenase (MDH)</td>
<td>Not specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Glutathione S-Transferase (GST-alpha)</td>
<td>Specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>F-protein (HPPD)</td>
<td>Highly specific</td>
<td>Hepatocellular regeneration</td>
</tr>
<tr>
<td>Arginase 1</td>
<td>Highly specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>alpha-fetoprotein</td>
<td>Specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>Regucalcin</td>
<td>Specific</td>
<td>Hepatocellular injury</td>
</tr>
<tr>
<td>alpha2,6-sialyltransferase (ST6gal)</td>
<td>Specific</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Not specific</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Colony stimulating factor receptor (CSF1R)</td>
<td>Not specific</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Paraoxonase 1 (PON1)/Prothrombin</td>
<td>Specific</td>
<td>Hepatocellular function</td>
</tr>
<tr>
<td>Leucocyte cell-derived chemotaxin2 (LECT2)</td>
<td>Not specific</td>
<td>Inflammation</td>
</tr>
</tbody>
</table>
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

Courtesy Jonathan Moggs, Novartis

ALT 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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Hepatology 54:1767
Standard liver tests: focus on liver only

Need to account for mechanistic background

HMGB1 and Cytokeratin 18
Mechanism based biomarkers

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

Apoptosis:
- **Keratin-18** – intermediate filament protein / structural integrity
- Is cleaved 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
Patients post acetaminophen overdose

Markers for inflammation, necrosis, and apoptosis

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

- 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
Diagnostic value of Glutathione S transferase $\alpha$ ($\alpha$GST)

- Glutathione S transferase $\alpha$ ($\alpha$GST):
  - Inducible phase II detoxification enzyme
  - Four isozymes of GST expressed in human and other mammals; $\alpha$GST is a liver specific dimer expressed in human hepatocyte cytosol
  - High concentration in centrilocular cells: may be more sensitive than ALT and AST
  - Half life $\sim 1$ 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 $\alpha$GST for liver monitoring
  - 150 healthy subjects (108 m, 42 f), age 18 - 60, BMI 18 - 32, duration 1-4 weeks
  - Key objectives:
    - Analyse correlation of $\alpha$GST levels with age, BMI, and aminotransferases at baseline, and with aminotransferases during treatment (active drug and placebo)
    - Characterize time profiles of $\alpha$GST as compared to ALT and AST
    - Explore to which extent $\alpha$GST 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
Initial, preliminary results of stage gate samples
CK18 full length and fragment, MCSF-R: DILI association

- Significant associations with DILI

*MCSF-R=CSF1R: cytokine receptor, controlling macrophage proliferation and function*
Initial, *preliminary* results of stage gate samples

*CK18 full length and fragment, MCSF-R: DILI (and gender?) association*

- MCSF-R: association with DILI *and* gender?
Initial, **preliminary** results of stage gate samples

**CK18 full length and fragment, MCSF-R: absence of age dependency**

- No associations with age
Initial, *preliminary* results of stage gate samples

CK18 full length and fragment, MCSF-R: absence of BMI dependency

- No associations with BMI
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
- Characteristic changes in serum proteome and metabolome expected
  - Mass spec and protein antibody array analyses of plasma samples ongoing
Healthy men and women (18-55 years) were treated with 4g acetaminophen/day for 7 days

- 17 subjects: responders (ALT >2.0 x baseline level)
- 15 subjects: intermediate responders (ALT 1.5-2.0 x baseline level)
- 18 subjects: non-responders (ALT <1.5)

Results
- Urine metabolite profiles prior or at start of treatment not predictive of DILI
- Urine profiles at day 5-6 (prior to raised ALT) could distinguish responders from non-responders
- Predictive metabolites include APAP and endogenous metabolites


Watkins Study: early predictive DILI markers
Additional benefit of serum proteomics and metabolomics

Courtesy Ina Schuppe Koistinen, AstraZeneca

- LC-MS based profiling for compound and endogenous metabolites
- Suspension bead protein array (antibody based)

**Metabolomics**

- Endogenous metabolites and protein profiles identified, that:
  - Predict ALT elevations at baseline (susceptibility markers)
  - Predict ALT elevations early during treatment

- Pathways involved
  - Pyruvate and glutamate metabolism at baseline
  - Cell death pathways activated at the time of ALT elevations
  - Overlap with ximelagatran candidate biomarkers

**Proteomics**

- Day 12
- Day 5 and 6
- Day 8 and 9
- Pre-dose
Conclusions

- Advanced safety biomarkers for prediction, detection, and assessment of drug-induced liver injury (DILI) are urgently needed.

- Due to the low incidence of DILI, large sample sizes across a range of different populations are required for clinical qualification of new markers.

- Large scale public private partnerships involving industry, academia, small to medium sized enterprises, and regulators such as the IMI SAFE-T consortium may be the most efficient way to successful biomarker qualification.

- A list of promising DILI biomarker candidates has been selected by the SAFE-T consortium for clinical qualification.

- Preliminary data on a subset of markers offer first insights into potential predictive and diagnostic value.

- Regulatory approval of new DILI biomarkers with defined contexts of use is expected by 2015.
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