Clinical biomarker qualification for drug-induced injury to kidney, liver and the vascular system within SAFE-T

Ina Schuppe Koistinen, AstraZeneca Safety Assessment
Scientific coordinator

Michael Merz, Novartis Institutes for BioMedical Research
Project coordinator
Three organs needing better clinical monitoring of drug-induced injuries:

**Kidney**: current standards increase only once 50-60% of kidney function is lost.

**Liver**: current standards are not sufficiently sensitive and specific and do not adequately discriminate adaptors from patients at high risk to develop liver failure.

IMI SAFE-T Consortium

Objectives

• To evaluate utility of safety BMs for monitoring DIKI, DILI and DIVI in humans.

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

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

• To gain evidence for how safety BMs may also be used in the diagnosis of diseases and in clinical practice
SAFE-T structure and deliverables

- Evidence-based decision making
- More reliable causality assessment
- Better mechanistic understanding
- Safer translation to clinical development
- Earlier and more specific signal detection
- Enhanced clinical monitoring

- Improved patient safety
- Reduced attrition rates
- Accelerated and safer approval of innovative medicines
SAFE-T biobank at ICCC in Barcelona

Workflow

WP2, WP3, WP4
(CLINICAL PARTNERS)

- patient / samples codes
- guidelines sample collection, handling shipment

WP5
(BM ASSAYS)

SAMPLE REQUEST

SAMPLE

SAMPLES
- biological sample
- sample information

WP6
(DATA ANALYSIS)

SAFE-T Consortium
Sample request approval

WP1
Regulatory requirements

SAMPLE REQUEST

Patient information for sample request

Sample information for sample request

Courtesy Teresa Padro, ICCC
Data capture, management, and analysis

Key challenge and key success for WP6

- Definition of user requirements, set up of process, and identification of suitable vendor significantly more complex than anticipated.
- Workable, cost-efficient solution established with IMI-JU support and in close collaboration with key SAFE-T work packages.
- Provides a solid basis for data and knowledge sharing with other IMI projects.

Courtesy Hannes Planatscher, NMI
Assay development and validation

- Assay validation process defined and implemented
- Assay development (80%) and low bar validation (75%)
- Concept of stage-gate cohorts implemented
- Testing ongoing
Biomarker qualification process

Elements and process flow

- Literature
- Databases
- SAFE-T sources

Select

- Healthy volunteers
- Patients with x-disease
- Patients with non-x disease
- Patients on x-toxic drugs

Select

Biomarker step 1 list
Evaluation
Biomarker step 2 list

Regulatory advice
Assay availability / development
Biomarker step 3 list

Exploratory phase
Assay / stat analysis / select specific+sensitive BMs
Biomarker step 4 list

Background variability
Thresholds (ROCs)

Confirmatory phase
Regulatory advice
Assay / stat analysis / select specific+sensitive BMs
Biomarker final list

Submit to health authorities
Regulatory approval

Q2 2009
Q1 2010
Q2 2011
Q2 2014
Identification of biomarker candidates

Separating the wheat from the chaff

From a long list of potentially interesting markers, 79 have been picked for further assessment in exploratory qualification studies.
DIKI biomarker qualification strategy

[Diagram showing the flow of the qualification strategy with decision points for variability in healthy subjects, response to DIKI, response to non-kidney disease, and response to non-DIKI kidney disease.}

Decision points include:
- High variability in healthy subjects: Drop
- Low variability in healthy subjects:
  - Good response to DIKI: Yes
  - Bad response to DIKI: Drop
- Response to non-kidney disease:
  - Yes: Drop
  - No:
    - Pathology?
    - Mechanism?
    - Disease severity?
    - Drug-relatedness?
    - Clinical outcome?
## DIKI biomarker candidates

<table>
<thead>
<tr>
<th>Type of biomarker</th>
<th>Biomarker name</th>
<th>Main significance</th>
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<tbody>
<tr>
<td><strong>Functional biomarkers</strong></td>
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<tr>
<td></td>
<td>Microalbumin</td>
<td>Marker of impaired proximal tubular re-absorption</td>
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<td></td>
<td>α-1 microglobulin</td>
<td>Marker of impaired proximal tubular re-absorption (and indirectly glomerular injury)</td>
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<td></td>
<td>Cystatin C</td>
<td>Evaluation of glomerular filtration rate (serum) Marker of impaired proximal tubular re-absorption (and indirectly of glomerular injury (urine))</td>
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<td></td>
<td>Retinol Binding Protein-4 (RBP-4)</td>
<td>Marker of impaired proximal tubular re-absorption</td>
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<td><strong>Tissue injury leakage markers</strong></td>
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<tr>
<td></td>
<td>N-acetyl-β-D-glucosaminidase (NAG)</td>
<td>Marker of proximal tubular injury</td>
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<td>Glutathione-S-transferase-α (GST-α)</td>
<td>Marker of proximal tubular injury</td>
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<td></td>
<td>Glutathione-S-transferase-π (GST-π)</td>
<td>Marker of distal tubular injury</td>
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<td></td>
<td>Liver-type fatty acid binding protein (L-FABP)</td>
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<td>Collagen IV</td>
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<td>Podocin</td>
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<td>Nephrin</td>
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<td>Aquaporin-2</td>
<td>Marker of collecting duct injury</td>
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<td></td>
<td>Calbindin D28</td>
<td>Marker of injury to distal regions of nephron and collecting ducts</td>
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<td><strong>Tissue injury response markers</strong></td>
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<tr>
<td></td>
<td>Kidney injury molecule-1 (KIM-1)</td>
<td>Marker of proximal tubular injury/regeneration</td>
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<td>Clusterin</td>
<td>Marker of tubular injury/regeneration (no apparent specific nephronal localization)</td>
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<td>Neutrophil gelatinase associated lipocalin (NGAL)</td>
<td>Marker of tubular (mainly proximal) injury</td>
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<td>Trefoil Factor 3 (TFF3)</td>
<td>Marker of proximal tubular injury</td>
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<td>Osteopontin</td>
<td>Marker of injury to distal regions of nephron</td>
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<td>Tissue inhibitor of metalloproteinase-1 (TIMP-1)</td>
<td>Marker of interstitial fibrosis and tubular injury</td>
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<td>Connective Tissue Growth Factor (CTGF)</td>
<td>Marker of interstitial fibrosis</td>
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<td>Interleukin-18 (IL-18)</td>
<td>Marker of inflammation</td>
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<td></td>
<td>Monocyte chemoattractant protein-1 (MCP-1)</td>
<td>Marker of inflammation</td>
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DIKI Exploratory phase: main studies

• Baseline studies
  • Healthy volunteer study
  • Chronic kidney disease study
  • Non-renal disease patient samples

• Renal injury studies
  – Proximal tubular damage studies
    • Cisplatin in cancer patients study
    • Contrast induced nephropathy study
  – Glomerular damage studies
    • Acute glomerulonephritis patient study

NOTE: Nephrotoxicity studies will be in patients receiving Standard of Care treatment
Cisplatin Study Design

Patients with cancer due to receive cisplatin chemotherapy as Standard of Care

Group A: patients with various cancers who are scheduled to start high dose cisplatin therapy.  
*N*=100

Group B: control patients with similar cancers treated with local radiotherapy or non-nephrotoxic drugs.  
*N*=20

Group C: non-treatment healthy volunteers.  
*N*=20
Strategies to enable the assessment of sensitive or prodromal biomarkers

- Biomarker
- Serum Creatinine
- Biopsy (GN)
- Time series + outcome
- Adjudication committee

Biomarker

Time

Biomarker

sCr

Time

# DILI biomarkers – status of assay development

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<th>Candidate biomarker</th>
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<td>albumin mRNA</td>
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<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
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<tr>
<td><strong>High mobility group box 1 (acetylated vs. non-acetylated)</strong></td>
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<td>Conjugated/unconjugated bile acids</td>
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<td><strong>High mobility group box 1 (acetylated vs. non-acetylated)</strong></td>
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<tr>
<td>ALT 1 &amp; 2, isoform specific</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><strong>Keratin 18 (caspase cleaved &amp; intact)</strong></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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<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>
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<td>LECT2</td>
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<tr>
<td><strong>Glutamate dehydrogenase (GLUD, GLDH)</strong></td>
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<tr>
<td>Purine nucleoside phosphorylase (PNP)</td>
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<td>Malate dehydrogenase (MDH)</td>
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<td>Sorbitol dehydrogenase (SDH)</td>
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<tr>
<td><strong>ALT1/2, isoform specific</strong></td>
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**Legend:**
- Ready for sample screening
- Ready for small sample sizes
- Optimization phase
- In development
- Development necessary

**Status:**
- ![status](#) - Ready for sample screening
- ![status](#) - Ready for small sample sizes
- ![status](#) - Optimization phase
- ![status](#) - In development
- ![status](#) - 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
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
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 Kiper, † 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
Patients post acetaminophen overdose (3)

HMGB1 as a potential prognostic marker

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
  – ALT assay developed at AZ measures human ALT isoforms (ALT1 & ALT2).

• Liver surgery study:
  – 12 patients undergoing open liver resection
  – Mean age 66.6, 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)

• Extreme Adventure race study
  – 39 participants, 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

Pre and post liver surgery and physical exercise

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

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)

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

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

• Challenge to design a qualification strategy for biomarkers of a preclinical toxicity that has no direct clinical correlate
  – In contrast to DILI (e.g., APAP and ALT) or DIKI (e.g., cisplatin and BUN/creatinine), there is no gold standard to perform “standard” ROC analyses for DIVI

• Most currently used biomarkers of vascular inflammation in the clinic are non-specific
  – Include markers of endothelial and smooth muscle cell damage
  – Result is that a panel of biomarkers is likely necessary

• Candidate biomarkers selected on basis of both preclinical and clinical association with vascular damage
  – Thus, a forward and reverse translation strategy is necessary
  – Interaction between PSTC and SAFE-T is important

• Overall, our strategy is to use surrogate patient populations presenting disease or drug-induced vascular damage.

Hypothesis: Similar histopathology between preclinical DIVI and vascular injury/disease in humans will lead to overlapping biomarker signatures
DIVI: Overall strategy (continued)

Parallel Reverse/Forward translation

Biomarker candidates of preclinical DIVI

Preclinical marker

Proof of translation studies

Proof of performance studies

Preclinical qualification studies

Clinical marker candidates

Damage from DIVI-drugs

Biomarker candidates of damaged vessels in man

Damage from drugs, immune-mediated diseases, atherosclerosis, radiation, etc
Biomarker candidates and assay status

Validated assays:
- IL-6
- CCL19
- VCAM
- ICAM3
- SAA
- CRP
- TGFb
- NO2
- TIMP1
- SELP
- ICAM1
- ELAM1
- CCL3
- CCL2
- ICAM3
- SAA
- CRP
- THBD
- SELP
- ICAM1
- ELAM1
- VEGF
- TGFb
- VCAM
- ICAM3
- SAA
- CRP
- THBD
- SELP
- ICAM1
- ELAM1
- VEGF
- TGFb
- VCAM
- ICAM3
- SAA
- CRP
- THBD
- SELP
- ICAM1
- ELAM1

In development:
- EDN1
- TAGLN
- CAV1
- EMPs
- ACE
- EMPs
- ACTA2
- ESM1
- THSP1
- SMTN
- CALD1

In validation:
- vWFpp
- CNN1
- vWF

Clinical routine parameters*
- Siemens biochemistry
- Siemens Immunology

* Use can be considered in the project if necessary

Mesoscale
Flow cytometry
Colorimetric
ELISA
Assay grouped in a multiplexed assay
To be determined
Preclinical studies to support DIVI

Several preclinical models were set-up by the ICCC and the TASMC:

- A rat model of intravital microscopy to assess the effect of DIVI on micro circulation (ICCC)
- A porcine model of coronary vasoactivity (ICCC)
- A murine model of atherosclerosis to study the effect of DIVI drug on pre-existing conditions

Additionally, two reverse translation were conducted:

- In rat treated with fenoldopan and CI-1044 (Pfizer)
- In rat treated with SK&F95654 (Sanofi)
Prospective studies

Coronary angiography patients
- Assessment of exploratory vascular markers in response to atherosclerotic lesions

Ballon angioplasty patients
- Assessment of exploratory vascular markers in response to acute endothelial injury

Vasculitides patients
- Assessment of exploratory vascular markers in acute and remission phase of:
  - Mixed cryoglobulinemia
  - Behçet's disease
  - Sjögren syndrome
  - Takayasu's disease

Patients treated with drugs associated with preclinical DIVI
- Assessment of exploratory vascular markers in response to administration of drug associated with preclinical DIVI in phase I-like studies
Biomarkers can discriminate patient populations

- Multivariate analysis of candidate biomarkers reveals separation of patient populations
- Results demonstrate proof of concept
Summary

• Consortium-based approach to safety biomarker qualification working with Regulatory Agencies and academic community

• 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

• Academic sites: **12 prospective clinical studies initiated**

• Inclusion of five additional academic partners has significantly increased capacity for DIKI and DILI qualification efforts without additional funding

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

• Initiated regulatory interactions via briefing meetings with EMA/FDA

• Established collaboration with Predictive Safety Testing Consortium (PSTC)
Next Steps

- Completion of exploratory studies
- Analysis of novel biomarker data and determine which are appropriate to test in confirmatory phase
- Design of confirmatory studies with Regulatory Agency advice
## Acknowledgements

*(Very incomplete) SAFE-T participant list, with team leaders*

**Total number of SAFE-T participants: >200!**

<table>
<thead>
<tr>
<th>Neus Prats</th>
<th>Heidrun Ellinger-Ziegelbauer</th>
<th>Fuat Firat</th>
<th>David Laurie</th>
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