SAFE-T Consortium Update

Safer and Faster Evidence-based Translation

Gerd A. Kullak-Ublick, Frances Hackman, Simon Kirby, Sif Ormarsdottir, Thierry Poynard, Michael Merz and SAFE-T investigators

Rachel Church, Paul Watkins and DILIN investigators
The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115003, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies’ in kind contribution.
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.


- Appropriate **DIKI, DILI and DIVI biomarkers** and methods qualified/supported by the EMA and FDA for use in medical product development

- **Database for human safety biomarkers** with a detailed characterization of clinical, individual and drug-specific factors in the context of drug-induced toxicities and diseases.

- **Biobank of human material**, obtained at different time points from patients enrolled in the clinical trials run by the consortium, to support future qualification of new biomarkers.
SAFE-T Objectives

- **Define a generic scientific qualification strategy** for translational safety biomarkers.

- **Develop and validate assays** for the quantification of biomarkers in clinical samples.

- **Select mechanistic biomarkers for DIKI, DILI, and DIVI** from current knowledge, different non-clinical discovery, pre-clinical qualification exercises and proprietary knowledge of the partners, based on their potential for translational use.

- **Qualify the translational biomarkers** in clinical settings for regulatory decision making in a ‘two step approach’: biomarker Proof of Translation (exploratory) studies, then biomarker Proof of Performance (confirmatory) studies.
The IMI funding model

- Samples
- Expertise (FTEs)
- (Cash)

1:1

Private Investment (€ 1 billion) in kind

EU Public Funding (€ 1 billion) cash

EFPIA

Pharma 1
Pharma 2
Pharma 3
Pharma 4
Pharma 5
Pharma 6

ACADEMIA
HOSPITALS
PATIENTS' ORGANISATIONS
SMALL AND MEDIUM-SIZED ENTERPRISES
REGULATORS
IMI SAFE-T Consortium

Academia

EfpiA

AstraZeneca  Novartis  Pfizer

Roche  Lilly  Bayer HealthCare  Bayer Schering Pharma

gsk  GlaxoSmithKline  Boehringer Ingelheim  sanofi aventis

Alimrall  AMGEN  Takeda

SMEs

FIRALIS  INTERFACE EUROPE  INTERVENTIONAL THERAPEUTICS

EKF Diagnostics  EDI  EPILOG

External contractors

SweTox  KOEHLER eClinical  ABU-CRO

Advisors

European Medicines Agency  SCIENCE MEDICINES HEALTH  FDA

Collaborators

PSTC  CRITICAL PATH INSTITUTE

IMI SAFE-T Consortium Update | Gerd Kullak-Ublick | June 7, 2017 | DILI Conference XVII | Session IV: Consortia
IMI SAFE-T research network

[Map showing the network of SAFE-T research network in Europe with cities and connections for 2009 and 2011 dates.]
Samples in SAFE-T consortium biobank

Serum, plasma und urine samples from > 11'000 patients across 19 different populations and healthy subjects
## IMI SAFE-T DILI marker panel

<table>
<thead>
<tr>
<th>Primary readout</th>
<th>Marker</th>
<th>Specific/additional readout</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leakage</strong></td>
<td>microRNA 122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glutamate dehydrogenase (GLDH)</td>
<td>Mitochondrial toxicity</td>
</tr>
<tr>
<td></td>
<td>Glutathione S-Transferase (GST-alpha)</td>
<td>Glutathione depletion?</td>
</tr>
<tr>
<td></td>
<td>Arginase 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sorbitol dehydrogenase (SDH)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total HMGB-1</td>
<td>Necrosis</td>
</tr>
<tr>
<td></td>
<td>Cytokeratin 18, full length protein</td>
<td>Necrosis</td>
</tr>
<tr>
<td><strong>Mechanism</strong></td>
<td>HMGB-1, hyperacetylated form</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td></td>
<td>Cytokeratin 18, caspase cleaved fragment</td>
<td>Apoptosis</td>
</tr>
<tr>
<td></td>
<td>Osteopontin</td>
<td>Inflammation, lymphocyte activation</td>
</tr>
<tr>
<td></td>
<td>Colony stimulating factor receptor (CSF1R) = MCSF-R</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td><strong>Regeneration</strong></td>
<td>Leucocyte cell-derived chemotaxin2 (LECT2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpha-fetoprotein</td>
<td></td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Paraoxonase 1 (PON1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conjugated/unconjugated bile acids</td>
<td>Cholestatic injury</td>
</tr>
</tbody>
</table>
Beyond serum ALT – exploratory circulating biomarkers for DILI

**Hepatocyte**

- **ALT**
- **HMGB1**
- **Keratin-18 (FL)**
- **miR-122**
- **Keratin-18 (CC)**
- **GLDH**

**Hepatocyte Injury**

**Necrosis**

**Immune Cell**

- **HMGB1**
- **M-CSF1**
- **HMGB1-Acetyl**

**Apoptosis**

**Mitochondrial Dysfunction**

**Immune Cell Activation**

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courtesy: Jonathan Moggs
## DILI sample selection

*by study, injury type, and causing agents*

### Study

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV (Tel Aviv)</td>
<td>193</td>
</tr>
<tr>
<td>Swiss DILI</td>
<td>28 DILI (32 total)</td>
</tr>
<tr>
<td>Prospective DILI</td>
<td>100 DILI (119 total)</td>
</tr>
<tr>
<td>Anti-TB</td>
<td>81</td>
</tr>
<tr>
<td>RA</td>
<td>92</td>
</tr>
<tr>
<td><strong>DILIN</strong></td>
<td><strong>166 DILI</strong></td>
</tr>
<tr>
<td>Liverpool APAP cohort</td>
<td>128</td>
</tr>
</tbody>
</table>

### Injury type

<table>
<thead>
<tr>
<th>DILI Type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestatic</td>
<td>12</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>91</td>
</tr>
<tr>
<td>Mixed</td>
<td>25</td>
</tr>
</tbody>
</table>

### Causative drugs

<table>
<thead>
<tr>
<th>DILI Drug Class</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP</td>
<td>19</td>
</tr>
<tr>
<td>APAP + NSAID</td>
<td>1</td>
</tr>
<tr>
<td>APAP + others</td>
<td>1</td>
</tr>
<tr>
<td>NSAID</td>
<td>4</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>33</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>7</td>
</tr>
<tr>
<td>Flupirtine</td>
<td>14</td>
</tr>
<tr>
<td>Anti-Tbc</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>46</td>
</tr>
</tbody>
</table>
Mechanism-based biomarkers

HMGB1 and Cytokeratin 18: immune activation and necrosis

ALT

Acet-HMGB1 (ng/ml)

Total Keratin 18 (U/L)

<table>
<thead>
<tr>
<th>Natural Log Value</th>
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</thead>
<tbody>
<tr>
<td>n = 123</td>
</tr>
<tr>
<td>n = 181</td>
</tr>
<tr>
<td>n = 121</td>
</tr>
<tr>
<td>n = 154</td>
</tr>
<tr>
<td>n = 113</td>
</tr>
<tr>
<td>n = 192</td>
</tr>
</tbody>
</table>
SAFE-T data:
Biomarker at baseline blood sample by causative drug

<table>
<thead>
<tr>
<th>Causative drug</th>
<th>APAP</th>
<th>flupirtine</th>
<th>amoxicillin</th>
<th>antibiotics</th>
<th>chemo</th>
<th>NSAID</th>
<th>other</th>
<th>HV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest ALT in APAP group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Line is the median, Diamond is the mean

n = 19  
n = 14  
n = 11  
n = 21  
n = 6  
n = 6  
n = 44  
n = 181
SAFE-T data:
Biomarker at baseline blood sample by causative drug

Total keratin 18 (U/L) (necrosis marker)

Total keratin 18 data for APAP-induced DILI already published by Liverpool group: SAFE-T data confirm findings for APAP and compare them with other causative drugs and healthy volunteers.
Macrophage Colony Stimulating Factor Receptor 1
Mechanism based biomarkers (immune activation)

MCSFR1 (ng/ml)

➢ differentiate severe idiosyncratic from intrinsic toxicity?
MCSFR1 in baseline blood sample according to whether Hy’s law criteria were fulfilled or not

Figure A15.9 - Boxplot of Baseline Biomarker Data by Hy’s Law at Baseline Studies: Protocol 3A, Swiss DILI and HV (Tel Aviv)

- MCSFR1 (ng/mL)
- Hy’s Law at Baseline (N = 31), No Hy’s Law at Baseline (N = 70), HV (N = 179)
- Line is the median, Diamond is the mean
- Hy’s Law is defined as ALT > 3*ULN and Total Bilirubin > 2*ULN

Source Dataset: biomarker.sas7bdat (dated 27th August 2015 09:35)
Run Date and Time of DILI Exploratory CoU Figure A15: 12OCT15:11:45:25
IMI SAFE-T DILI Exploratory Analyses
miR-122 in baseline blood sample according to whether Hy’s law criteria were fulfilled or not.
Novartis xxx study: microRNA-122 results

ALT and miR122 (Copies/µl) over time by subject; subjects with ALT > 3x ULN only; one y-axis scale per parameter
Bile Acids

Mechanism based biomarkers

Taurochenodeoxycholic acid (nmol/L)

2-OH deoxy bile acid (nmol/L)
Date: July 25, 2016

ATTN: Safer and Faster Evidence-based Translation (SAFE-T) Consortium
Fanny Gaby, Firalis
Gerd Kullak-Ublick, Novartis
Angelika Hoenlinger, Novartis

Subject: Letter of Support for Drug-Induced Liver Injury (DILI) Biomarker(s)

Dear Safe-T Consortium,

We are issuing this Letter of Support to the SAFE-T Consortium to encourage the further development and exploratory use of:

- Cytokeratin 18 (CK-18)
- Total and hyperacetylated high mobility group protein B1 (HMGB1)
- Osteopontin
- Macrophage colony-stimulating factor 1 receptor (CSF1R)

alone or in combination as soluble monitoring biomarkers to assess the risk of progression of drug-induced liver injury (DILI) in patients in whom an initial DILI diagnosis has been established based on elevations of the standard biomarkers alanine aminotransferase (ALT) alone or in combination with total bilirubin (TBIL) as a clinical safety assessment in clinical trials in a drug development context.
Letter of support for drug-induced liver injury (DILI) biomarker

Recommendations

Recommendation 1:
Clear and unconditional support to encourage further research is given for the biomarker candidates included in the proposed Context-of-use statement B. It appears that the most promising results have been achieved within this context, and the direction for further research can be defined more easily: The parameters hyperacetylated HMGB1, Osteopontin, Total Keratin 18 and MCSFR1 have potential as clinical DILI biomarkers to identify and assess the risk of progression in patients with an established diagnosis of DILI based on current standard criteria. Sponsors may choose to incorporate these parameters into their clinical trials to anticipate early a risk for progression of hepatocellular injury to severe DILI in patients in whom an initial DILI diagnosis has been established based on elevations of the standard marker ALT alone or in combination with TBIL.
Letter of support for drug-induced liver injury (DILI) biomarker

**Recommendation 2:**
Further promising results have been achieved for the biomarkers explored in the proposed context of use statement C, which also describes a field where the direction for further research can be more easily defined. However, it does not most obviously relate to the development of new chemical entities for the treatment of diseases, unless a similar “intrinsic” toxicity as for the well-established compound paracetamol (acetaminophen) will have been established for a new chemical entity.

The parameters *total HMGB1, total and caspase-cleaved keratin 18, miR-122 and GLDH* have the potential to be used as clinical safety biomarkers that sponsors may choose to incorporate in clinical trials with compounds having suspected intrinsic liver toxicity in order to potentially improve the early (within 24 hours) prediction of the occurrence of liver injury.
SAFE-T’s new liver safety biomarkers

Supporting early detection, prognosis, and mechanistic understanding

- Nine new liver safety biomarkers supported by EMA and/or FDA for exploratory use in clinical drug development:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HMGB1</td>
<td>Mechanism (necrosis), prognosis</td>
</tr>
<tr>
<td>Hyperacetylated HMGB1</td>
<td>Mechanism (immune activation), prognosis</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Prognosis</td>
</tr>
<tr>
<td>Total Keratin 18</td>
<td>Mechanism (necrosis), prognosis</td>
</tr>
<tr>
<td>Caspase-cleaved keratin 18</td>
<td>Mechanism (apoptosis), prognosis</td>
</tr>
<tr>
<td>MCSFR1</td>
<td>Mechanism (immune activation), prognosis</td>
</tr>
<tr>
<td>miR-122</td>
<td>Detection, mechanism (hepatocyte leakage)</td>
</tr>
<tr>
<td>GLDH</td>
<td>Detection, mechanism (mitochondrial injury)</td>
</tr>
<tr>
<td>SDH</td>
<td>Detection</td>
</tr>
</tbody>
</table>
Summary

- Qualitatively, SAFE-T and DILIN acute DILI patients show similar levels of new biomarkers in the biosample taken at baseline (i.e. when DILI is still evident).

- SAFE-T has APAP and healthy volunteer control groups to compare with.

- 14 cases of flupirtine in the SAFE-T cohort stand out for several biomarkers.

- DILIN data offer outcome analysis, predictive biomarkers include acetylated HMGB1, osteopontin and MCSFR1.
Acknowledgements

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In particular:

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- Simon Kirby, Frances Hackman: WP3 statistics
- Kaidre Bendjama, Mike Lawton; WP4 lead
- Thomas Schindler; WP5 lead
- Martin Schumacher; WP6 lead
- Axel Kretschmer, Teresa Padro, Lina Badimon; WP7 lead
- Nicole Schneiderhan-Marra; managing entity, WP8 lead
- Marc Loher; WP9 lead
- Isabelle Clavier, Catherine Lunven, Joachim Tillner; XWP

PSTC:

In particular:

- Liz Walker
- John-Michael Sauer
- Nicholas King
- Rich Miller
- Jeff Lawrence
- Doug Keller
- Shelli Schomaker

DILIN:

In particular:

- Paul B. Watkins
- Rachel Church
The Translational Safety Biomarker Pipeline*

Building on SAFE-T’s achievements and learnings

*Proposal currently being updated under leadership of Pfizer