Safety Biomarkers:
Opportunities and challenges in drug discovery and development

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Outline

• Reasons for attrition in drug R&D
• What types of safety biomarkers do we need to develop innovative drugs to treat diseases?
• Biomarker qualification within the SAFE-T consortium
# Attrition in drug R&D

The various toxicity domains have been ranked first by contribution to products withdrawn from sale, then by attrition during clinical development.

<table>
<thead>
<tr>
<th>Phase</th>
<th>‘Nonclinical’</th>
<th>Phase I</th>
<th>Phase I-III</th>
<th>Phase III/Marketing</th>
<th>Post-Marketing</th>
<th>Post-Marketing</th>
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</thead>
<tbody>
<tr>
<td>Information:</td>
<td>Causes of attrition</td>
<td>Serious ADRs</td>
<td>Causes of attrition</td>
<td>ADRs on label</td>
<td>Serious ADRs</td>
<td>Withdrawal from sale</td>
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<tr>
<td>Sample size:</td>
<td>88 CDs stopped</td>
<td>1,015 subjects</td>
<td>82 CDs stopped</td>
<td>1,138 drugs</td>
<td>21,298 patients</td>
<td>47 drugs</td>
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<tr>
<td>Cardiovascular:</td>
<td>27%</td>
<td>9%</td>
<td>21%</td>
<td>36%</td>
<td>15%</td>
<td>45%</td>
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<tr>
<td>Hepatotoxicity:</td>
<td>8%</td>
<td>7%</td>
<td>21%</td>
<td>13%</td>
<td>0%</td>
<td>32%</td>
</tr>
<tr>
<td>Haematology/BM:</td>
<td>7%</td>
<td>2%</td>
<td>4%</td>
<td>16%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td>Nervous system:</td>
<td>14%</td>
<td>28%</td>
<td>21%</td>
<td>67%</td>
<td>39%</td>
<td>9%</td>
</tr>
<tr>
<td>Immunotox; photosensitivity:</td>
<td>7%</td>
<td>16%</td>
<td>11%</td>
<td>25%</td>
<td>34%</td>
<td>2%</td>
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<td>23%</td>
<td>5%</td>
<td>67%</td>
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<td>Reprotox:</td>
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<td>10%</td>
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<td>Musculoskeletal:</td>
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<td>1%</td>
<td>28%</td>
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<tr>
<td>Respiratory:</td>
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<td>0%</td>
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<td>Renal:</td>
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<td>Genetic tox:</td>
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<td>Carcinogenicity:</td>
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<td>0%</td>
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<tr>
<td>Other:</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Adapted from Redfern WS et al. SOT 2010
Translational Safety Biomarkers: Scenarios for use

The ultimate goal is to provide drug R&D with a toolbox of qualified safety biomarkers that perform well for a drug candidate in animal studies and can be used for the same drug candidate to predict and monitor clinical safety.
Pharmacogenomic biomarkers

Presentation by Eleni Aklillu

Sim and Ingelman-Sundberg, TiPS 2011, 32(2):72-
Drug-induced kidney injury biomarkers

Adapted from
Bonventre et al 2010 Nat. Biotech 28:436
Drug-induced cardiovascular injury

Presentation by Ruth Roberts

Both functional and structural safety biomarkers needed
Drug-induced liver injury (DILI)

What types of new DILI biomarkers are needed?

• New biomarker(s) should be more *sensitive* and *specific* for detection of DILI and give mechanistic information about the injury.

• Predicitive DILI biomarkers that signal liver injury before ALT has increased in patients.

• Prognostic DILI biomarkers that follow the development of injury and signal if a patient is recovering or developing acute liver failure.

• Susceptibility biomarkers for patient stratification to select patients at risk
Watkins Study: Discovery of predictive DILI biomarkers

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 x baseline level)

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

Collaboration with Paul Watkins, Univ North Carolina

Biomarker discovery: Suspension bead protein arrays

• Screening procedure
  - 32 patients, only pre-dose samples, categorized into responders and non-responders
  - 3 800 antibodies immobilized on the Luminex Flexmap 3D-system

• Targeted analysis
  - Development of DILI array
  - 16 responders, 16 non-responders
  - all time points
  - 382 serum samples

Collaboration with SciLife Lab, Mattias Uhlén, Peter Nilsson, Jochen Schwenk, Marcus Gry (AZ)
Results Protein profiling: Screening

- 3800 antibodies in array
- 16 responders, 16 non-responders, day 1 (pre-dose)
- Two different MVA methods show clear separation between the groups, overlapping protein lists
- 90 antibodies selected for targeted DILI array

Protein pattern identified that predict ALT elevations prior to treatment with acetaminophen
Results Protein profiling: DILI Array

Protein pattern identified that predict ALT elevations

- prior to treatment with acetaminophen
- early during treatment with acetaminophen
Key challenges for SBM qualification

- Large number of preclinical studies needed linking biomarker to histopathology
- Translatability across species incl humans
- Lack of access to human histopathology
- Multitude of patient populations need to be included (background variability)
- Large number of biomarker candidates require substantial sample volumes
- Key target responses, i.e. specific ADRs, suitable and accessible for qualification are overall very rare or difficult to mimick in animals
- Regulators require broad scientific consensus for SBMs qualified for regulatory decision-making

Qualification cannot be achieved by one laboratory/company alone
Qualification of SBM requires collaboration
SAFE-T (Safer and faster evidence-based translation)

Objective
- To qualify new specific and sensitive safety biomarkers for drug-induced kidney, liver and vascular injuries to improve safety assessment during drug development

- 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 safe approval of innovative medicines

Project coordinator: Michael Merz (Novartis)
Scientific co-ordinator: Ina Schuppe Koistinen (AstraZeneca)
11 Pharma, 8 academic, 4 SME partners  Budget 35.7 mio Euro  Duration: 5 years (start June 09)
SAFE-T: Clinical biomarker qualification process

1. **Select** Biomarker step 1 list
2. **Evalutation**
3. **Select** Biomarker step 2 list
4. **Exploratory phase**
   - Assay availability / development
   - Assay / stat analysis / select specific + sensitive BMs
   - Biomarker step 3 list
   - Biomarker step 4 list
5. **Confirmatory phase**
   - Assay / stat analysis / select specific + sensitive BMs
   - Biomarker final list
6. **Regulatory advice**
   - Background variability
   - Thresholds (ROCs)
7. **Qualification**
8. **Submit to health authorities**
9. **Regulatory approval**

**Sources:**
- Literature
- Databases
- SAFE-T sources

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

**Timeline:**
- Q2 2009
- Q1 2010
- Q2 2011
- Q2 2014
Identification of biomarker candidates

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

**DILI**
- Albumin mRNA
- Microglobulin precursor mRNA
- Albumin 122 conjugated/unconjugated bile acids
- High mobility group box 1 (HMGB1)
- Cytokeratin 18 (KRT18)
- Alpha fetoprotein (AFP)
- Arginase 1
- Colony stimulating factor receptor (CSF1R)
- F-protein (HPPD)
- Glutathione S transferase
- Alpha (GSTα)
- Leukocyte cell-derived chemotaxin 2 (LECT2)
- ST3Gal 1
- Osteopontin
- Ratios paraoxonase (PON)
- Prothrombin
- Regucalcin (RGN)
- ALT1/2
- Glutamate dehydrogenase (GLUD, GLDH)
- Malat dehydrogenase (MMDH)
- Purine nucleoside phosphorylase (PNP)

**DIVI**
- E-Selectin
- P-Selectin
- Thrombomodulin
- Caveolin 1
- H1-Caldesmon
- SM22/Transgelin
- Smooth muscle alpha actin
- Circulating endothelial cells
- Albumin mRNA
- Microglobulin precursor (Ambp) mRNA
- Micro RNA 122
- Conjugated/unconjugated bile acids
- High mobility group box 1 (HMGB1)
- Cytokeratin 18 (KRT18)
- Alpha fetoprotein (AFP)
- Arginase 1
- Colony stimulating factor receptor (CSF1R)
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- Malat dehydrogenase (MMDH)
- Purine nucleoside phosphorylase (PNP)

**DIKI**
- Microalbumin/Albumin
- α-1 microglobulin
- Cystatin C
- Urea/creatinine
- Retinol binding protein (RBP-4)
- N-acetyl-β-D-glucosaminidase (NAG)
- Glutathione-S-transferase-α (GST-α)
- Glutathione-S-transferase-ι (GST-ι)
- Liver-type fatty acid binding protein (L-FABP)
- Collagen IV
- Podocin
- Aquaporin-2
- Galbindin D28
- Kidney injury molecule
- Clustern
- Neutrophil gelatinase associated lipocalin (NGAL)
- Trefoil Factor 3 (TFF3)
- Osteopontin
- Tissue inhibitor of metalloproteinase-1 (TIMP-1)
- Connective Tissue Growth Factor (CTGF)
- Interleukin-11 (IL-11)
- Monocyte chemoattractant protein-1 (MCP-1)
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
# SAFE-T DILI biomarkers

<table>
<thead>
<tr>
<th>Candidate biomarker</th>
<th>Status</th>
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<tbody>
<tr>
<td>miRNA 122</td>
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</tr>
<tr>
<td>albumin mRNA</td>
<td></td>
</tr>
<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
<td></td>
</tr>
<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
<td>Optimization phase</td>
</tr>
<tr>
<td>Conjugated/unconjugated bile acids</td>
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</tr>
<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
<td>Optimization phase</td>
</tr>
<tr>
<td>ALT 1 &amp; 2, isoform specific</td>
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</tr>
<tr>
<td>F-protein (HPPD)</td>
<td>In development</td>
</tr>
<tr>
<td>Arginase 1</td>
<td></td>
</tr>
<tr>
<td>Keratin 18 (caspase cleaved &amp; intact)</td>
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</tr>
<tr>
<td>Alpha fetoprotein (AFP)</td>
<td>Development necessary</td>
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<tr>
<td>Regucalcin (RGN)</td>
<td></td>
</tr>
<tr>
<td>Glutathione S-Transferase (GST-alpha)</td>
<td></td>
</tr>
<tr>
<td>ST6gal I</td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
<td></td>
</tr>
<tr>
<td>Colony stimulating factor receptor (CSF1R)</td>
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</tr>
<tr>
<td>Paraoxonase 1 (PON1)</td>
<td></td>
</tr>
<tr>
<td>Prothrombin</td>
<td></td>
</tr>
<tr>
<td>LECT2</td>
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</tr>
<tr>
<td>Glutamate dehydrogenase (GLUD, GLDH)</td>
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<tr>
<td>Purine nucleoside phosphorylase (PNP)</td>
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<tr>
<td>Malate dehydrogenase (MDH)</td>
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<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
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</tr>
<tr>
<td>ALT 1/2, isoform specific</td>
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</tr>
</tbody>
</table>

**Legend:**
- Green: Ready for sample screening
- Yellow: Ready for small sample sizes
- Orange: Optimization phase
- Red: In development
- Purple: Development necessary
Cytokeratin 18 (CK18)

- Intermediate filament protein expressed primarily in epithelial cells

- Abundantly expressed in the liver – biomarker of hepatocyte injury in plasma

- Human CK18 ELISA developed by Peviva:
  - **M65**: full-length CK18 (necrosis + apoptosis)
  - **M30**: caspase-cleaved CK18 (apoptosis)

- Both M65 and M30 increase in plasma during liver fibrosis and steatosis

- M30 is elevated in plasma during various forms of carcinomas (breast, colon, lung, testicular, pancreatic, head/neck, GI)

Courtesy Petra Thulin, AZ
Results Cytokeratin 18 APAP HV study

- Both M65 and M30 increased significantly in the responders from day 8 and onwards.
- The elevation at day 8 was 1.3 fold both for ALT and M30 but 2.0 fold for M65.
- The maximum increase for ALT was 2.4-fold and 2.9 fold for M65 (day 12).
- The ALT elevation remained high after APAP was withdrawn, whereas M65 declined.
Results miRNA-122 in APAP HV study

- Total RNA was extracted from 50 μl serum from day 2-13
- miR-122 specific qRT-PCR was performed
- miR-122 levels were normalised to a synthetic miRNA added to the samples before RNA extraction (c-el-39)
- miR-122 increased significantly in the responders from day 8 and remained elevated.
- The maximum increase for miR-122 was 4.0 fold change at day 11

*Courtesy Petra Thulin, AZ*
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
SAFE-T key achievements so far

- Collaboration within the consortium is excellent
- Generic qualification strategy defined
- Biomarker candidates prioritised, assay development well advanced, first data generated
- Central biobank for sample storage, 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: 6 studies
  - Submitted or under preparation: 5 studies
- Initiated regulatory interactions via briefing meetings with EMA/FDA
- Established collaboration with Predictive Safety Testing Consortium (PSTC)
SAFE-T participants

Advisors

SMEs

Academia

Collaborators
Many organ systems lack SBM to monitor drug-induced injuries