Safety Biomarkers: Opportunities and challenges in drug discovery and development

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EUROTOX 2012, Stockholm
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

<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>
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<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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<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>
<th>Toxicity Domain</th>
<th>Cardiovascular</th>
<th>Hepatotoxicity</th>
<th>Haematology/BM</th>
<th>Nervous system</th>
<th>Immunotoxicity</th>
<th>Gastrointestinal</th>
<th>Reprotox</th>
<th>Musculoskeletal</th>
<th>Respiratory</th>
<th>Renal</th>
<th>Genetic tox</th>
<th>Carcinogenicity</th>
<th>Other</th>
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The various toxicity domains have been ranked first by contribution to products withdrawn from sale, then by attrition during clinical development.

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

Presentation by Joe Keenan

Adapted from Bonventre et al 2010 Nat. Biotech 28:436

Diagram showing kidney anatomy and highlighted biomarkers.

- Proximal tubules
  - Kim-1
  - Clusterin
  - NGAL
  - GST-α
  - β2-microglobulin
  - α1-microglobulin
  - NAG
  - Osteopontin
  - Cystatin C (urinary)
  - Neprilysin-1
  - RBP
  - IL-1β
  - HGF
  - Cyr61
  - NHE-3
  - Exosomal fetuin-A
  - L-FABP
  - Albumin

- Distal tubules
  - Osteopontin
  - Clusterin
  - GST-α/π
  - NGAL
  - H-FABP
  - Calbindin D28

- Collecting duct
  - Calbindin D28

- Loop of Henle
  - Osteopontin
  - NHE-3

- Glomerulus
  - Total protein
  - Cystatin C (urinary)
  - β2-microglobulin
  - α1-microglobulin
  - Albumin

- Proximal tubules
  - Cyclosporine

- Papilla
- Cortex
- Medulla
- Pelvis
- Ureter
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.

- Predictive 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 mimic 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 co-ordinator: 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

Select

- Literature
- Databases
- SAFE-T sources

Select

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

Samples

Biomarker step 1 list
Evaluation
Biomarker step 2 list

Regulatory advice

Explorative phase

- Assay availability / development
- Biomarker step 3 list
- 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

Qualification

Submit to health authorities

Regulatory approval

Q2 2009
Q1 2010
Q2 2011
Q2 2014

R&D | Innovative Medicines | Global Safety Assessment
Identification of biomarker candidates

From a long list of potentially interesting markers, 79 have been picked for further assessment in exploratory qualification studies.
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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<td>albumin mRNA</td>
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<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
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<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
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<tr>
<td>Conjugated/unconjugated bile acids</td>
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<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
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<tr>
<td>ALT 1 &amp; 2, isoform specific</td>
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<td>F-protein (HPPD)</td>
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<td>Arginase 1</td>
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<td>Keratin 18 (caspase cleaved &amp; intact)</td>
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<td>Alpha fetoprotein (AFP)</td>
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<td>Regucalcin (RGN)</td>
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<td>Glutathione S-Transferase (GST-alpha)</td>
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<td>ST6gal I</td>
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<td>Osteopontin</td>
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<td>Colony stimulating factor receptor (CSF1R)</td>
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<td>Paraoxonase 1 (PON1)</td>
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<td>Prothrombin</td>
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<td>LECT2</td>
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<td>Glutamate dehydrogenase (GLUD, GLDH)</td>
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<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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<td>ALT1/2, isoform specific</td>
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- **Ready for sample screening**
- **Ready for small sample sizes**
- **Optimization phase**
- **In development**
- **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

ALT

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D12 = 2.4 fold

M65

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D12 = 2.9 fold

M30

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D11 = 1.5 fold

• 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 ul 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
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

Academia

SMEs

Advisors

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