Predictive safety biomarkers in non-clinical development

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Merck Serono Research
Agenda

• Why safety biomarkers?

• Strategy at Merck Serono

• What has been achieved so far:
  – Acute hepatotoxicity (PhD project)
  – Sub-acute hepatotoxicity and nephrotoxicity (PredTox)
  – FDA/EMEA biomarker qualification – nephrotoxicity

• Outcomes, benefits and challenges
Why new safety biomarkers?

- Traditional animal models not always predictive of toxicity in humans
  - Drug attrition often related to toxicity/safety
  - Current endpoints indicate mostly late stage effects only
- Potential to speed up non-clinical drug safety
- Increased understanding of toxicity mechanisms
- Early translational information for early clinical safety
- Earlier decision making and de-risking compounds
- Reducing late phase failures
What is needed:
New technology development

• Data sharing and openness between pharmaceutical companies on models used in drug development and experimental design

• Increased interaction with external knowledge partners (academia, service providers, consortia) like public–private partnerships using omics approaches in drug safety assessment:
## Public–private partnerships using omics approaches in drug safety assessment

<table>
<thead>
<tr>
<th>Consortium title</th>
<th>Website</th>
<th>Technologies represented(^a)</th>
<th>Species and study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEBS programme of the US NIEHS</td>
<td><a href="http://cebs.niehs.nih.gov/">http://cebs.niehs.nih.gov/</a></td>
<td>Tx, Px</td>
<td>Rat/mouse; primary focus on liver</td>
</tr>
<tr>
<td>Japanese Toxicogenomics Project</td>
<td><a href="http://www.tgp.nibio.go.jp/index-e.html">www.tgp.nibio.go.jp/index-e.html</a></td>
<td>Tx</td>
<td>Rat plus <em>in vitro</em>; primary focus on liver</td>
</tr>
<tr>
<td>FDA and BG medicine liver toxicity biomarker study</td>
<td><a href="http://www.fda.gov/CDER/livertox/presentations">www.fda.gov/CDER/livertox/presentations</a></td>
<td>Tx, Px, Mx</td>
<td>Rat; focus on liver</td>
</tr>
<tr>
<td>COMET project</td>
<td><a href="http://www.metabolomics.net/index.aspx?ID=64360">www.metabolomics.net/index.aspx?ID=64360</a></td>
<td>Mx</td>
<td>Rat; (liver and kidney toxins)</td>
</tr>
<tr>
<td>HESI Genomics Committee</td>
<td><a href="http://www.hesiglobal.org/">www.hesiglobal.org/</a></td>
<td>Tx</td>
<td>Multiple projects, rat and <em>in vitro</em>; kidney, heart and genotoxicity</td>
</tr>
<tr>
<td>InnoMed PredTox</td>
<td><a href="http://www.innomed-predtox.com">www.innomed-predtox.com</a></td>
<td>Tx, Px, Mx</td>
<td>Rat (focus on liver and kidney tissues, plus blood/urine)</td>
</tr>
</tbody>
</table>
Types of biomarkers

- **Surrogates biomarkers**
  - Predictive
    - In vitro (*Predict-IV*)
    - Indicates onset of lesion early on

- **Mechanistic biomarkers**
  - explain the mechanism by which certain events occurred
  - Understand a particular histopathological outcome
Novel biomarker problem

- Diversity of:-
  - Species
    - Rodent specific or human specific
      - Lack of translation across tox species!!
  - Compound
    - New biomarkers are compound/class specific
    - Lack of –ve/+ve samples
  - Sample
    - Too few sample types represented
Toxicity Biomarker Strategy at Merck

Use full range of biomarkers to support *in vivo* studies

- Clinical pathology
- IHC
- Protein marker (multiplex in tissues, blood, urine)
- Gene expression marker (multiplex and/or global)
- Nephrotoxicity (FDA/EMEA qualified)
- *In vitro* hepatotoxicity
- Cytokines (Luminex multiplex)
- New gene expression marker sets
  - mechanistic or for identification/prediction

Use translational biomarker for clinical monitoring
Current applications for Merck Serono

1. Acute hepatotoxicity (PhD project)

2. Sub-acute hepatotoxicity and nephrotoxicity (PredTox)

3. FDA/EMEA qualified nephrotoxicity biomarkers
1. Current hepatotoxicity biomarkers

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphatase (ALP)
  - work well in the liver and kidney when the toxicity well established
- Not good enough …
  - Hepatotox major reason for drug failure
  - Species specificity – human relevance

Therefore, need biomarkers for altered cell function/signaling during early structural alterations / reversible

New novel biomarkers (Ozer et al., 2008):- C-PATH / qualification process?
  Serum F Protein, Arginase I, GSTa
Genomic prediction model

**Aim**

Establish predictive screening test for acute hepatotoxicity

*Can focused Illumina Microarray be used for class prediction of compounds?*

- 12 model compounds
- *In vivo rat – single i.p. dose*
- 550 genes
Results: Cross validation (Classification) of compound (Supervised method)

Classifier: Support Vector Machine - categories: hepatotoxicity/non-hepatotoxicity - Test set fraction: 0 (leave one out method)
Unknown compounds

Model tested with 2 „unknowns‟:

- LCB3343  -ve (24h)  Yes
- Bromobenzene  +ve (24h)  Yes

- Identification of 64 safety marker genes for class prediction supports the establishment of a predictive hepatotoxicity model
- Early acute “biomarker” set??
2. PredTox EU FP6 Project Summary

• List of Biomarker Candidates from EWG–I:

  **Liver Hypertrophy (LH)**
  – Urinary increase in phenylacetylglycine
    • Potential metabolite marker candidate for ER proliferation
  – Urinary decrease in trigonelline
    • Potential marker candidate for mobilization of oxidative stress signaling

• List of Biomarker Candidates from EWG-II:

  **Bile Duct Damage / Necrosis (BDN)**
  – Unconjugated vs. conjugated bile acids in urine (and serum)
    • Mechanism-based markers for hepatocyte vs. bile duct damage
  – Thiostatin, NGAL (Lipocalin-2), and Clusterin protein in urine and/or serum
    • Non-invasive, general diagnostic markers of tissue injury and/or inflammation – non specific
List of Biomarker Candidates from EWG-III:

**Nephrotoxicity (Proximal Tubule Damage - PTD)**

- mRNA (tissue): specific mRNA signature
  - complement system genes or specific cytoskeleton components, seven mechanism-based markers deregulated before histopathological changes
  - Highly expressed and very specific gene Osteoactivin
- Protein (tissue): Decrease of kidney Glycine amidinotransferase protein in 3 PTD studies
  - Potential marker candidate for proximal tubule damage
- Protein and mRNA (tissue): very specific increase of Coro1a (Coronin 1a) and CapG (capping protein actin filament, gelsolin-like)
  - New specific marker candidates for tissue damage
3. Nephrotoxicity

- Drug induced nephrotoxicity
- Biomarker localisation
- Kidney Luminex xMAP assay (RBM-Merck/EMD)

Conclusions

- The urinary kidney BMs (Kim-1, Albumin, Total Protein, β2-Microglobulin, urinary albumin, urinary retinol binding protein 3 and urinary Cystatin C) are considered acceptable in the context of non-clinical drug development for the detection of acute drug-induced nephrotoxicity, either tubular or glomerular with associated tubular involvement.
- They provide additional and complementary information to BUN and serum creatinine to correlate with histo-pathological alterations considered to be the gold standard.
- It is recognised that it is worthwhile further exploring in early clinical trials the potential of Kim-1, Albumin, Total Protein, β2-Microglobulin, Urinary clusterin, Urinary retinol binding protein 3, and urinary Cystatin C as clinical BMs for acute drug-induced kidney injury. Until further data are available to correlate the BMs with the evolution of the nephrotoxic alterations, and their reversibility, their general use for monitoring nephrotoxicity in clinical setting cannot be recommended.
- Incremental Qualification Potential in non-clinical context:
  Further expand data on the correlation between the BMs and the evolution and reversibility, of acute kidney injury. Also, further expand the knowledge on species-specificity.
- Ways to best implement these biomarkers in a further development program, can be discussed on a case by case basis in the context of the new EMEA qualification advice (see http://www.ema.europa.eu/pdfs/human/biomarkers/7289408en.pdf).
Drug Induced Nephrotoxicity

• Kidney is a major “underestimated” target of drug toxicity
  – most toxicity is sub clinical
  – kidney has great capacity to recover

• Long term: -
  – Once renal function is compromised renal failure is progressive

• Standard metrics, serum creatinine and blood urea nitrogen levels
  – insensitive, non-specific
  – change only after significant injury and with substantial delay
  – 50% of renal function may be lost before serum creatinine changes significantly

• New biomarkers - developed by Novartis (originally) together with Merck and Rules Based Medicine
  – Through Critical Path Initiative (FDA)
Biomarker Localization

Proposed exploratory biomarkers

- **Glomeruli (1):**
  - β-2-microglobulin
  - Podocin

- **Proximal Tubules (2):**
  - Alpha –GST (Glutathione-S-transferase)
  - KIM-1 (Kidney injury molecule-1)
  - Clusterin
  - TFF3
  - Osteopontin
  - β-2-microglobulin
  - Calbindin D28
  - NAG (N-acetyl-b-D-glucosaminidase)
  - TIMP-1 (Tissue inhibitor of metalloproteinases-1)

- **Loop of Henle (3):**
  - Osteopontin

- **Distal Tubules (4):**
  - mu GST
  - KIM-1
  - Clusterin
  - Cytostatin c
  - Osteopontin
  - TIMP-1

- **Vascular markers:**
  - Cysteine-rich protein-61 (CYR61)
  - VEGF (Vascular endothelial growth factor)

- **General Toxicity:**
  - EGF (Epidermal

June 2008
7 prioritised for FDA/EMEA submission (+ Albumin and total urinary protein)
Kidney Luminex xMAP assay
(RBM - MERCK/EMD)

1. b-2-Microglobulin
2. Calbindin
3. Clusterin
4. Cystatin-C
5. Glutathione S-Transferase-alpha (GST-a)
7. Neutrophil Gelatinase Associated Lipocalin (NGAL)
8. Osteopontin
9. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)
10. Vascular Endothelial Growth Factor (VEGF)

Now available from Merck
(www.rbmmaps.com)

Human panel now available – developed based on translational approach (via RBM)

Will be established internally for 14day tox rat studies (case-by-case)
- In vivo rat study planned
- Sub-acute biomarker search
- PhD programme
- CCNet/BioRN BMBF project application

27.02.2009
Phil Hewitt - NIM 16.04.09
Summary – NCD safety biomarkers

- Good predictive biomarker signature for acute *in vivo* hepatotoxicity
  - 64 gene biomarker signature
  - need different “fingerprint” for repeat-dosing (BioRN BMBF)
- *In vitro* assessment very promising - hepatocytes (Hrach PhD thesis)
  - Too many genes for screening assay (~700)?
  - But - 92.5% correct prediction!
- FFPE gene and protein expression
  - Several methods available and assessed
  - Initial comparison to fresh frozen gene expression promising
- Nephrotox biomarkers
  - Internal validation ongoing
  - BioRN BMBF / Predict-IV EU projects
Critical Issues

Regulatory acceptance

- Validation vs qualification
- Proactive interaction between Pharma and regulators

Quality control essential (GLP!)

Assay development – translational (to other tox species as well as human)

- Gene or protein
- Urine/blood or tissue (for NCD)

Translational biomarkers

- Currently only “forward” translation
- Need better “backward” translation
  - FFPE ‘omics
Scientific Benefits

Predicting toxicity in early animal and laboratory studies will

- improve drug safety screening
- allow to select the safest drug candidate for development
- help to select initial human dose with more accuracy
- Allowing continued development because of hitherto un-monitorable toxicities
- provide better monitoring for side effects prior to clinical trials
- REACH / cosmetics directive and 3Rs
Predicting toxicity in early animal and laboratory studies will

- Stop candidate development earlier / correctly
- Reduce attrition rate and costs
- Prevent late stage/post-marketing withdrawals

FDA’s *Critical Path Initiative* addresses Tox issues as mission critical topic
Acknowledgement

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“The most difficult step in the adoption of novel safety biomarkers is the merging of the information these tests provide into key decision-making steps in the drug development process”.

Dr F. Goodsaid, FDA (2004)