Immunoassays in Multiplex for Personalized Medicine

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Drug Safety

(Critical Path Initiative)

Predictive Safety Testing Consortium

- Bristol-Myers Squibb Company
- Roche
- GlaxoSmithKline
- Pfizer
- Lilly
- AstraZeneca
- Merck
- Amgen
- Johnson & Johnson
- Boehringer Ingelheim
- Novartis
- Schering-Plough
- Wyeth

RBM
RULES BASED MEDICINE
Antibody based Biomarker assays

- FDA Critical Path Initiative
- Predictive Safety Testing Consortium (PSTC)
- 7 urinary biomarkers for drug induced kidney injury submitted for FDA approval

*Nature Biotechnology 28, May 2010*
Antibody based Biomarker assays

- Rat Nephrotoxicity Panel
- Rules Base Medicine / Luminex Array

1. β-2 Microglobulin
2. Calbindin
3. Clusterin
4. Cystatin-C
5. Epidermal Growth Factor (EGF)
6. Glutathione S-Transferase-alpha (GST-α)
7. Glutathione S-Transferase-pi (GST-Π)
8. Kidney injury molecule 1 (KIM1)
9. Neutrophil Gelatinase Associated Lipocalin (NGAL)
10. Osteopontin
11. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)
12. Vascular Endothelial Growth Factor (VEGF)
Kidney Toxicity Standard

Proximal Tubules
- Alpha –GST
- KIM-1
- Clusterin
- Osteopontin
- ß-2-microglobulin
- Calbindin d28
- NAG
- TIMP-1

Distal Tubules
- mu GST
- KIM-1
- Clusterin
- Osteopontin
- TIMP-1

Glomeruli
- ß-2-microglobulin
- Podocin

http://www.uic.edu/classes/bios/bios100/lecturesf04am/kidney01e.jpg

The Innovative Medicine Initiative
Safer & Faster Evidence Based Translation project

Qualification of new specific and sensitive safety biomarkers for drug-induced

Kidney

Liver

Vascular injury to improve safety of drug development
Drug safety: room for improvement
The economic perspective

- Around 90% of compounds entering clinical development fail

- 30% of these failures are due to clinical safety and toxicology

Kola et al. (2004), Nat Rev Drug Discovery ; 3: 711-15
The Innovative Medicine Initiative
The SAFE-T project:

**Select**
- Literature
- Databases
- SAFE-T sources

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

**Biomarker step 1 list**
- Evaluation

**Biomarker step 2 list**

**Exploratory phase**
- Assay availability / development
- Biomarker step 3 list
  - Assay / stat analysis / select specific+sensitive BMs
- Biomarker step 4 list
  - Assay / stat analysis / select specific+sensitive BMs

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

**Regulatory advice**
- Background variability
- Thresholds (ROCs)
- Qualification

**Submit to health authorities**

**Regulatory approval**
The Innovative Medicine Initiative
The SAFE-T project:

List of Biomarker candidates

Biomarker step 1 list

List of Biomarker candidates

Biomarker step 2 list

Technical Validation

Exploratory phase

Clinical Validation

Confirmatory phase

Regulatory advice

Assay / stat analysis / select specific+sensitive BMs

Background variability

Thresholds (ROCs)

Regulatory advice

Assay / stat analysis / select specific+sensitive BMs

Qualification

Submit to health authorities

Regulatory approval

Matheis et al., DDT, 2011, 16 (13-14)
DILI biomarker candidates selected for qualification

- Drug Induced Liver Injury is a rare event, sample availability is limited

<table>
<thead>
<tr>
<th>Serum or Plasma Marker</th>
<th>Assays</th>
<th>Liver Specificity</th>
<th>Human Data</th>
<th>Pathology</th>
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</thead>
<tbody>
<tr>
<td>miRNA 122</td>
<td>RNA / QPCR</td>
<td>highly specific</td>
<td>yes</td>
<td>heptocellular damage</td>
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<tr>
<td>albumin mRNA</td>
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<tr>
<td>Microglobulin precursor (Ambp) mRNA</td>
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<tr>
<td>High mobility group box 1 (acetylated vs. non-acetylated)</td>
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<td>only in tissue</td>
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<td>F-protein (HPPD)</td>
<td>LC-MS</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>Regucalcin (RGN)</td>
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<td>Glutathione S-Transferase (GST-alpha)</td>
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<td>specific</td>
<td>yes</td>
<td>inflammation</td>
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<tr>
<td>ST6gal I</td>
<td></td>
<td>specific</td>
<td>yes</td>
<td>heptocellular damage</td>
</tr>
<tr>
<td>Osteopontin</td>
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<td>not specific</td>
<td>yes</td>
<td>inflammation</td>
</tr>
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<td>Colony stimulating factor receptor (CSF1R)</td>
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<td>not specific</td>
<td>yes</td>
<td>inflammation</td>
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<tr>
<td>LECT2</td>
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<td>not specific</td>
<td>yes</td>
<td>inflammation</td>
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<tr>
<td>Paraoxonase 1 (PON1)</td>
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<td>not specific</td>
<td>yes</td>
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<td>Prothrombin</td>
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<td>Glutamate dehydrogenase (GLUD, GLDH)</td>
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<tr>
<td>Sorbitol dehydrogenase (SDH)</td>
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</tbody>
</table>

Composite disease markers to be assessed in addition: ActiTest™, Fibrotest™, SteatoTest™
SAFE-T

- **DIKI**
  - 21 biomarkers listed for evaluation in SAFE-T
  - For some markers results available from preclinical experiments (PSTC)
  - All biomarkers are now tested with samples from clinical studies

- **DIVI**
  - Not much known about potential biomarkers
  - 35 potential biomarkers were chosen for evaluation

→ Identified biomarkers will not only be applied in drug development, but might also be used for personalized medicine.