Biomarker of Drug Induced Kidney Injury Qualification for Regulatory Decision Making

Jacky Vonderscher,
Head of Exploratory Development in Europe
IOM/FDA, April 23th
Silver Spring, MD
1. Background of FDA Novartis CRADA Project

2. Study Design

3. Results from Cisplatin Study

4. Results from all CRADA Studies

5. Way Forward
Current Tests to Monitor Drug-induced Nephrotoxicity

- Serum Creatinine
- Blood Urea Nitrogen (BUN)
- Glomerular Filtration Rate (GFR)

- Test renal function, not integrity
- Damage has already occurred
- No changes of kidney function tests until 2/3 loss of nephrons
- Compensation can cause additional long-term damage
- Blood concentrations of Creatinine and BUN also influenced by production
An „Ideal“ Biomarker

- Early
- Indicative after active damage
- Sensitive
- Correlates with severity
- Accessible in peripheral tissue
- „Analytically“ stable in tissue
- Bridges across species
- Known mechanism and limitations
- Differentiates molecular effects
- Localizes damage

Can a single biomarker accommodate this list?
Do we need a biomarker signature?
Novartis / FDA CDER
Renal Safety Biomarker CRADA: Objectives

A Cooperative Research And Development Agreement between Novartis and FDA/CDER:

1. To identify a process by which biomarkers for safety can be qualified for use in regulatory decision-making
2. To qualify kidney safety biomarkers for regulatory decision making in pre-clinical settings
3. To propose a path forward for the qualification in man
From Exploratory Biomarkers to Qualified Biomarkers

**Observational or Exploratory Biomarker***

For internal decision making

**Probable Valid Biomarker***

Appropriate for regulatory decision making

**Known Valid Biomarker***

Sharing Experience, Consortia, Agencies

Exploratory studies

Qualification project:
Demonstrate significance of results in a test system with well-established performance

*See [http://www.fda.gov/cber/gdlns/pharmdtasub.pdf](http://www.fda.gov/cber/gdlns/pharmdtasub.pdf)
FDA Qualification Process

From Federico Goodsayd and Felix Frueh in Pharmacogenomics 2006, 7(5), 773-782

Biomarker Discovery & Method Development

Study Protocol Proposal

Dose Range Finding Study

Validation Study

Validation Study Report

Cross-Validation Consortium

Exploratory Biomarker

Probable Valid Biomarker

Known Valid Biomarker

From Federico Goodsayd and Felix Frueh in Pharmacogenomics 2006, 7(5), 773-782
Renal Safety CRADA main studies

2-3 weeks rat studies with 8 nephrotoxicants and 2 hepatotoxicants

96 animals per test compound

In-Life Data
- Food consumption
- Observations
- Body weight

Clin. Chem.
- Blood
- Urine
- NAG

Pathology
- Microscopic findings
- Macroscopic
- Organ weight

Multiform. PCR
- Kidney
- Liver

Multiplex ELISA
- Kidney
- Liver
- Urine
- Plasma

Data Analysis
- Classical parameters
- Gold standard: Histopath
- New parameters
In-life Studies – Generic Study Design

- 5 days
- 3 days
- 1 day

4 termination time points
x 4 doses (ctl, low, mid, high)
x 6 animals per group

96 animals per compound

- single/multiple dose
- 2 / 3 weeks
## Renal Safety CRADA: Han Wistar rat studies

2-3 weeks studies with 8 nephrotoxicants and 2 hepatotoxicants

### 8 Nephrotoxicants

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>x</td>
<td>(x)</td>
<td></td>
<td>Lysosomal phospholipidosis</td>
</tr>
<tr>
<td>Puromycin</td>
<td>x (2\textsuperscript{nd})</td>
<td>x</td>
<td></td>
<td>Damage to podocytes</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>x</td>
<td></td>
<td></td>
<td>Oxidative stress (free radicals)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>x (2\textsuperscript{nd})</td>
<td>x</td>
<td></td>
<td>Oxidative stress to glom. filtr. membrane</td>
</tr>
<tr>
<td>Furosemide</td>
<td>x</td>
<td></td>
<td></td>
<td>Mineralization</td>
</tr>
<tr>
<td>Lithium carbonate</td>
<td>x</td>
<td>(x)</td>
<td>x</td>
<td>Influences formation of intracellular cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>x</td>
<td>(x)</td>
<td>(x)</td>
<td>Direct DNA alkylation of DNA, Ox. stress</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>x</td>
<td>(x)</td>
<td></td>
<td>Complex (vasoconstrict., calcification...)</td>
</tr>
</tbody>
</table>

### 2 Hepatotoxicants

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mode of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-Naphthysothiocyanate (ANIT)</td>
<td>Cholangitis</td>
</tr>
<tr>
<td>Methapyrilene</td>
<td>Hepatocarcinogen (upon chronic treatment)</td>
</tr>
</tbody>
</table>
Selection of 15 markers for kidney safety monitoring

- **Proximal Tubule**
  - GST-α
  - b2-Microglobulin
  - NAG
  - Kim-1
  - Lipocalin-2
  - Timp-1
  - Clusterin
  - EGF
  - (Osteopontin)

- **Glomerulus**
  - Podocin
  - b2-Microglobulin (indirect)
  - Cystatin C

- **Loop of Henle**
  - β Osteopontin

- **Distal Tubule**
  - GST-μ/π
  - Osteopontin
  - Calbindin D28
  - Timp-1
  - Clusterin
  - EGF

- **Collecting Duct**
  - (Calbindin D28)
Observed Histopathology

1. Tubular Cell Degeneration
2. Tubular Cell Regeneration
3. Tubular Cell Alterations
4. Intratubular Casts
5. Tubular Dilatation
6. Glomerular Alteration
7. Juxtaglomerular Apparatus Hypertrophy
8. Pelvis Dilatation
9. Inflammation
10. Fibrosis
11. Concentric Lamellar Bodies
12. Urothelial hypertrophy-hyperplasia
## Current Standard Methods: Histopathology

- Quantifiable? related to Biomarker?
- 166 Localizations of lesions possible (grade 0-5)
- Integration by two levels in molecular processes (16 possible processes)

<table>
<thead>
<tr>
<th>Processes</th>
<th>Lesion Types</th>
<th>Kidney Structural Elements / Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular Cell Degeneration</td>
<td>Necrosis</td>
<td>No precise localization possible&lt;br&gt;Prox. convoluted tubule (PCT, s1-s2)&lt;br&gt;Thick descending tubule (s3)&lt;br&gt;Loop of Henle&lt;br&gt;Thick ascending tubule&lt;br&gt;Distal convoluted tubule&lt;br&gt;Collecting duct</td>
</tr>
<tr>
<td>Apoptosis</td>
<td></td>
<td>No precise localization possible&lt;br&gt;Prox. convoluted tubule (PCT, s1-s2)&lt;br&gt;Thick descending tubule (s3)&lt;br&gt;Loop of Henle&lt;br&gt;Thick ascending tubule&lt;br&gt;Distal convoluted tubule&lt;br&gt;Collecting duct</td>
</tr>
<tr>
<td>Tubular Cell Regeneration</td>
<td>Basophilia</td>
<td>No precise localization possible&lt;br&gt;Prox. convoluted tubule (PCT, s1-s2)&lt;br&gt;Thick descending tubule (s3)&lt;br&gt;Loop of Henle&lt;br&gt;Thick ascending tubule&lt;br&gt;Distal convoluted tubule&lt;br&gt;Collecting duct</td>
</tr>
<tr>
<td>Mitosis increase</td>
<td></td>
<td>No precise localization possible&lt;br&gt;Prox. convoluted tubule (PCT, s1-s2)&lt;br&gt;Thick descending tubule (s3)&lt;br&gt;Loop of Henle&lt;br&gt;Thick ascending tubule&lt;br&gt;Distal convoluted tubule&lt;br&gt;Collecting duct</td>
</tr>
</tbody>
</table>
• 79 different types of localized lesions in kidney reported
Preliminary Results
Cisplatin

- Biomarkers of Tubular Necrosis/Apoptosis
  - Did we produce the expected pathology
  - Biomarker Concentrations vs Histopath
  - Quantitative assessment
Cisplatin: Tubular Necrosis, Apoptosis
Serum Creatinine (fold change)
Cisplatin: Tubular Necrosis, Apoptosis

BUN (fold change)
Cisplatin: Tubular Necrosis, Apoptosis
Urinary Kim-1 (fold change)

Logarithmic Scale!
Cisplatin: Tubular Necrosis, Apoptosis
Urinary Clusterin (fold change)

Logarithmic Scale!
Quantitative Assessment

• Control Animals:
  – Non-dosed or heptatoxicant-dosed
  – Absence of specific lesion (grade 0)

• Diseased Animals:
  – Non-dosed, hepatotoxicant-dosed, nephrotoxicant dosed
  – Presence of specific lesion (>grade 0)

• Assessment of Performance
  – ROC Analysis: Are under Curve (AUC) of Receiver Operating Characteristics
    False positive rate versus false negative rate
Cisplatin: Tubular Necrosis / Apoptosis

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>True Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>27 Diseased</td>
</tr>
<tr>
<td>0.55</td>
<td>17 Controls</td>
</tr>
</tbody>
</table>

Area Under Curve:
Random = 0.5
Creatinine = 0.53

1-Specificity False Positives
Cisplatin: Tubular Necrosis / Apoptosis

Area Under Curve:

Random = 0.5
Creatinine = 0.53

27 Diseased
17 Controls
Cisplatin: Tubular Necrosis / Apoptosis

Area Under Curve:
- Random = 0.5
- Creatinine = 0.53
- BUN = 0.62

27 Diseased
17 Controls
Cisplatin: Tubular Necrosis / Apoptosis

Area Under Curve:

Random = 0.5
Creatinine = 0.53
BUN = 0.62
Kim-1 = 0.99

27 Diseased
17 Controls
Cisplatin: Tubular Necrosis / Apoptosis

Area Under Curve:
- Random = 0.5
- Creatinine = 0.53
- BUN = 0.62
- Kim-1 = 0.99
- Clusterin = 0.93

27 Diseased
17 Controls
All 10 Studies

- 8 nephrotoxicants, 2 hepatotoxicants
- Biomarkers of Tubular Necrosis/Apoptosis
- Quantitative assessment
All Studies: Tubular Necrosis: Proximal + Non-localized

Area Under Curve:
- Random = 0.5
- Creatinine = 0.83
- BUN = 0.81
- Kim-1 = 0.95
- Clusterin = 0.93

78 Diseased
291 Controls
All Studies: Glomerular Alteration / Damage

Area Under Curve:

Random = 0.5

Creatinine = 0.52

Urinary Protein = 0.86

Urinary Cystatin C = 0.91

Urinary b2-Microglobulin = 0.89

41 Diseased

291 Controls

<table>
<thead>
<tr>
<th></th>
<th>1-Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
</table>
1. To identify a process by which biomarkers for safety can be qualified for use in regulatory decision-making

- Published

2. To qualify kidney safety biomarkers for regulatory decision making in pre-clinical settings

- Urinary KIM-1: ROC AUC = 0.95, Proximal Tubular necrosis
- Urinary Cystatin C: ROC AUC = 0.91, Glomerular Alteration
- A panel of biomarkers is critical to cover key renal dysfunctions
- Planned Submission FDA/EMEA July 12th, 07

3. To propose a path forward for the qualification in man

- Goal of Kidney Working Group of the Predictive Safety Testing Consortium (C-Path Institute)
Acknowledgements

Novartis Pharma
Overall Project Responsibility / Project Management / Data Integration / Signatures

CRO: Centre International de Toxicologie, Evreux, France
Rat Studies / In-Life / Clinical Chemistry / Urinalysis / Histopathogy
Sébastien Leuillet, Bernard Palatte

CRO: Biolytix, Witterswill, Switzerland
RT-PCR Methods and Samples analysis
Peter Brodmann, Dominik Moor

Havard Medical School, Boston, USA
KIM-1 material : Prof. Joseph Bonventre and Dr. Vishal Vaidya

CRO: RulesBased Medicine, Austin, Texas, USA
Multiplexed Protein ELISA assays and samples analysis
Jim Mapes

FDA
Federico Goodsaid, Felix Frueh, Courtney Harper, Joe Hackett, Bea Droke, David Jacobson-Kram ... many others