Qualification of New Safety Biomarkers of Organ Damage, Injury Response, and Dysfunction: Lessons from the Kidney

Frank D. Sistare
Merck and Co., Inc.
On behalf of the C-Path PSTC NWG and FNIH BC

March 24, 2011, Silver Spring, MD
FDA/AASLD/PhRMA 2011 DILI Meeting
“The story of the new kidney biomarkers will be of great interest, although the liver is a different beast than the kidney. Our pioneering leap forward from just using bilirubin for liver injury/dysfunction took place in 1955 with the addition of rapid spectrophotometric determination of transaminase activities in serum, and the combined compound biomarker of (ALT&TBL) elevation is now our gold standard for detection of liver injury, but we still can't predict what will happen.”

(Personal communication from John Senior, CDER, FDA.)
Additional translational safety biomarkers are needed to enable drug development by:

1. …enhancing safety monitoring of patients in early clinical trials for toxicities seen in animal studies that are of questionable human relevance, and

2. …reducing decision-making ambiguity on clinical trials with data that provide greater diagnostic insight over conventional biomarkers alone, allowing improved patient prognosis and greater understanding of drug action.
Nephrotoxicity is a serious problem for early drug development and current biomarkers for the detection of acute kidney injury are inadequate.

- Candidate compounds in drug development can cause histopathological lesions in the kidney in animals at doses and times where no measurable changes in serum creatinine (sCr) and/or blood urea nitrogen (BUN) are detected.
- Sometimes, such lesions are found in the kidney in a single species, or human irrelevant mechanisms are suspected.
- Such drug candidates are often abandoned in an effort to not expose patients in clinical trials to potential risk because they cannot be confidently monitored.
  - This leads to delays in development timelines for patients with significant medical needs due to inappropriate loss of drug candidates.

**Proposed project deliverable:** qualify a new set of biomarkers that outperform sCr and BUN for monitoring early onset and reversal of mild kidney injuries.
### Summary of “Fit for Purpose” Claims and Decisions

<table>
<thead>
<tr>
<th>Urinary Biomarker</th>
<th>Rat Kidney Pathologies</th>
<th>Clinical</th>
<th>Supporting Published Evidence</th>
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<tbody>
<tr>
<td></td>
<td>Can Outperform BUN &amp; Serum Cr</td>
<td>Monitor Glomerular Pathology</td>
<td>Monitor Tubular Pathologies (Necr., Degen., Dilatat’n, Regen.)</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Total Protein</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>KIM-1</td>
<td>✓</td>
<td></td>
<td>✓</td>
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<tr>
<td>Albumin</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Clusterin</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Trefoil Factor 3</td>
<td>✓</td>
<td></td>
<td>✓</td>
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</table>
**Hypothesis:**

New translational kidney safety biomarkers will:
1) report injuries to different segments of the nephron,
2) respond earlier and be more sensitive than BUN and sCr,
3) report on dysfunction, AND damage, AND histopathologic injury response processes (e.g., degeneration, regeneration, dilatation, etc.)
4) inform patient prognosis,
5) enable drug development.

**Next-generation biomarkers for detecting kidney toxicity**

Joseph V Bonventre, Vishal S Vaidya, Robert Schanoner, Peter Feig & Frank Dieterle

*NATURE BIOTECHNOLOGY* VOLUME 28 NUMBER 5 MAY 2010 pp 436 - 440

*Ten manuscripts in this issue on the submission of these biomarker data*
Example Study: Carbapenem A – Rat Kidney Biomarker Performance Assessment

- **Kim-1**: Graph showing fold change of Kim-1 with different animal samples.
- **Serum Creatinine**: Graph showing fold change of serum creatinine with different histopathological grades and control.
- **LCN-2**: Graph showing fold change of LCN-2 with different animal samples.
- **Clusterin**: Graph showing fold change of clusterin with different animal samples.

Legend:
- Untreated Control
- Treated Grade 0
- Treated Grade 1
- Treated Grade 2
- Treated Grade 3
- Treated Grade 4
Additional Studies

Kim-1

Fold change

Proximal Tubule Composite score
Mesoscale Composite score

- Untreated Control
- Treated Grade 0
- Treated Grade 1
- Treated Grade 2
- Treated Grade 3
- Treated Grade 4
- Treated Grade 5
Additional Studies

LCN-2

Fold change

Untreated Control
Treated Grade 0
Treated Grade 1
Treated Grade 2
Treated Grade 3
Treated Grade 4
Treated Grade 5

Proximal Tubule Composite score
Mesoscale Composite score

Cisplatin  Carbapenem  Cyclosporin  Thioacetamide  Gentamycin  HCB  D-serine  NPAA  Propyl  Adr.
Additional Studies

Clusterin

Fold change vs. Proximal Tubule Composite score and Mesoscale Composite score

- Untreated Control
- Treated Grade 0
- Treated Grade 1
- Treated Grade 2
- Treated Grade 3
- Treated Grade 4
- Treated Grade 5
Example Composite ROC Analysis: Tubular Biomarkers Outperform Serum Creatinine and BUN

Merck Data Shown: 9 studies, 264 total animals (179 injured, 85 injury-free)
Composite ROC Analysis: Glomerular Biomarkers Outperform Serum Creatinine and BUN

Area Under Curve:
- Cystatin C = 0.91
- B2-Microglob. = 0.89
- Tot. Protein = 0.86
- BUN = 0.80
- Creatinine = 0.52
- Random = 0.5

41 Damaged
289 Controls
Recovery Study: 150 mg/kg Carbapenem IV x 3 Days Followed by 15 Days Recovery

Concurrent Control Rat Kidney

Degeneration

Necrosis

Treatment Day 2

Regeneration

Necrotic epithelial cells within tubular lumens

Day 8 (recovery day 5)

Regeneration

Day 18 (recovery day 15)
Recovery Study: 150 mg/kg Carbapenem IV x 3 Days Followed by 15 Days Recovery
Recovery Study: 150 mg/kg Carbapenem IV x 3 Days Followed by 15 Days Recovery

V = Vehicle, T = Treated, N&D = Necrosis & Degeneration, R = Regeneration
Recovery Study: 150 mg/kg Carbapenem IV x 3 Days Followed by 15 Days Recovery

V = Vehicle, T = Treated, N&D = Necrosis & Degeneration, R = Regeneration
EMA & FDA Favorable Review Decisions

Following assessment, both regulatory agencies came to the conclusions that:

• the renal biomarkers submitted *were acceptable in the context of non-clinical drug development* for detection of acute drug-induced renal toxicity;

• the renal biomarkers provide additional and complementary information to the currently available standards;

• *the use of renal biomarkers in clinical trials is to be considered on a case-by-case basis* in order to gather further data to qualify their usefulness in monitoring drug-induced renal toxicity in man.
FNIH BC projects undergo a comprehensive development process to advance from concept to fully executable and funded project.

1. **EC/SC, RFA/RFP or External Submission**
   - Initial Idea or Concept
   - Scientific merit
   - Pre-competitive
   - Feasibility
   - Initial funding scan

2. **Steering Committee**
   - Approved Project
   - Protocol
   - Resources
   - Intellectual property
   - Data sharing
   - Timelines/milestones
   - Budget
   - Human subjects
   - Privacy
   - Legal review

3. **Steering Committee/Project Team**
   - Project Plan
   - Final QA/QC
   - Funding

4. **Executive Committee (and Funders)**
   - Approved Project
   - Contracts
   - Project management

5. **Project Team**
   - Launch

---

Approval by the Biomarkers Consortium Executive Committee on August 16, 2010.
Anticipated $3.25 M clinical study start date is June 2011.
• Advance regulatory acceptance for clinical applications of new “fit for purpose” renal safety biomarkers

• Inform the utility of new biomarkers to outperform sCr and BUN for monitoring safety from acute renal tubule injury with compounds dosed to relevant clinical exposures in Phase 1 and 2 clinical trials

• Provide practical thresholds of changes in these biomarkers that signify stopping criteria to halt or modify dosing

• This project will complement the clinical work proposed by the European based Innovative Medicines Initiative (IMI) SAFE-T Consortium, a portion of which will also focus on kidney safety
The clinical kidney BM project will pursue an efficient “learn and confirm” strategy

• Retrospective analysis samples:
  – Samples from malignant mesothelioma patients treated with cisplatin and meeting established criteria for AKI (50% rise of sCr)
  – A limited set of data on 16 urine biomarkers from 33 patients treated with cisplatin, carboplatin, or gemcitabine
  – ClinXus/ Jasper Observational Longitudinal Study – 80 patients to establish baseline variability characteristics for the biomarkers
  – Comparative assay performance data across 3 commercial providers
  – Set statistical and clinically meaningful thresholds for each biomarker; formulate adjudication rules for the second prospective phase

• Prospective analysis: 2 clinical trials
  – Observational Study to Evaluate Biomarkers of Cisplatin Nephrotoxicity (2 sites, 150 patients out of which 50 controls)
  – Observational Study to Evaluate Biomarkers of Aminoglycoside Nephrotoxicity (2 sites, 150 subjects out of which 50 controls)
Two clinical trials are being pursued

The two drugs selected by the Project Team are both known to cause injuries to the kidney tubule

150 patients, blood and urine

**Aminoglycosides**
(in patients with cystic fibrosis=CF)

**Primary Site**
The USC
PI: Paul Beringer

**Secondary site**
Un. of Minnesota
PI: Jordan Dunitz

150 patients, blood and urine

**Cisplatin**
(in patients with head and neck cancer)

**Primary Site**
MD Anderson
PI: Abdulla Sallahudeen

**Secondary site**
Dana Farber
PI: Sus Waiker
## Proposed Biomarkers of Acute Renal Damage to be Evaluated

<table>
<thead>
<tr>
<th>Functional Biomarkers</th>
<th>Proposed Functional Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Small quantities filtered by glomerulus and efficiently reabsorbed by tubular epithelium.</td>
</tr>
<tr>
<td>β2Mic</td>
<td>Glomerular damage protein overload inhibits tubular reabsorption from lumen</td>
</tr>
<tr>
<td>Serum Cystatin C</td>
<td>Functional measure of glomerular filtration and tubular reabsorption.</td>
</tr>
<tr>
<td>Urinary Cystatin C</td>
<td>Glomerular damage yields protein overload that inhibits tubular reabsorption from lumen</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Functional marker of glomerular filter integrity</td>
</tr>
<tr>
<td>RBP 4</td>
<td>Freely filtered by glomerulus and efficiently reabsorbed by tubular epithelium</td>
</tr>
<tr>
<td><strong>Damage/ Leakage Markers</strong></td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td>Brush-border enzyme released when damage occurs to tubular epithelium</td>
</tr>
<tr>
<td>GGT</td>
<td>Tubular epithelium cell membrane disruption releases GGT from cytosol</td>
</tr>
<tr>
<td>GSTα / GSTπ</td>
<td>Tubular epithelium cell membrane disruption and cytosol leakage</td>
</tr>
<tr>
<td><strong>Injury Response Markers</strong></td>
<td></td>
</tr>
<tr>
<td>KIM-1</td>
<td>Tubular epithelium dedifferentiation and regenerative repair response</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Regenerative repair response present in many renal cell types including tubular epithelium</td>
</tr>
<tr>
<td>IL-18</td>
<td>Tubular epithelium protein reflecting initiation of apoptotic cascades</td>
</tr>
<tr>
<td>L-FABP</td>
<td>Anoxia/ ischemia signal in tubular epithelium and potential oxidative damage signal</td>
</tr>
<tr>
<td>NGAL (Lipocalin 2)</td>
<td>Distal tubule inflammation &amp; rescue signal to sequester iron, limit damage, promote survival</td>
</tr>
<tr>
<td>Osteoactivin</td>
<td>Proposed role in attenuating degeneration, and potentially contributing to fibrosis</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Expressed in TAL and DCT, may limit oxidative stress and ischemia, and assist regeneration</td>
</tr>
<tr>
<td>Trefoil Factor 3</td>
<td>Decrease in concentration removes cellular maturation signaling, allowing dedifferentiation</td>
</tr>
<tr>
<td>Uromodulin (Tamm-Horsfall)</td>
<td>Host defense factor binds pathogens, toxins, inhibits proteases</td>
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</table>
The Current Members of the Project Team:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scott Adler, M.D.</td>
<td>Astra Zeneca</td>
<td>industry</td>
</tr>
<tr>
<td>William Baer, Ph.D.</td>
<td>ClinXus</td>
<td>nonprofit</td>
</tr>
<tr>
<td>Melanie Blank, M.D.</td>
<td>FDA</td>
<td>government</td>
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<td>James Chung, M.D., Ph.D.</td>
<td>Amgen</td>
<td>industry</td>
</tr>
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<td>academia</td>
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<td>Eli Lilly</td>
<td>industry</td>
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<td>nonprofit</td>
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<td>Peter Feig, M.D.</td>
<td>Merck</td>
<td>industry</td>
</tr>
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<td>Lloyd Haskell, Ph.D.</td>
<td>J&amp;J</td>
<td>industry</td>
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<td>NIDDK/NIH</td>
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<td>Merck</td>
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<td>Irene Nunes, D.O.</td>
<td>Merck Research Labs</td>
<td>industry</td>
</tr>
<tr>
<td>Frank Sistare, Ph.D.</td>
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</tr>
<tr>
<td>Norman Stockbridge, M.D.</td>
<td>FDA</td>
<td>government</td>
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<tr>
<td>Stefan Sultana, M.D.</td>
<td>Pfizer</td>
<td>industry</td>
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<tr>
<td>Aliza Thompson, M.D.</td>
<td>FDA</td>
<td>government</td>
</tr>
<tr>
<td>Maria Vassileva, Ph.D.</td>
<td>FNIH</td>
<td>FNIH Program Manager</td>
</tr>
<tr>
<td>Elizabeth Walker, Ph.D.</td>
<td>PSTC, CPI</td>
<td>nonprofit</td>
</tr>
<tr>
<td>Marc Walton, M.D.</td>
<td>FDA</td>
<td>government</td>
</tr>
<tr>
<td>David Warnock, M.D.</td>
<td>University of Alabama</td>
<td>academia</td>
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**Project PIs**

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<thead>
<tr>
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<tbody>
<tr>
<td>Paul Beringer, M.D.</td>
<td>USC</td>
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</tr>
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<td>University of Minnesota</td>
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<td>academia</td>
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<tr>
<td>Sushrut Waikar, M.D.</td>
<td>Harvard University</td>
<td>academia</td>
</tr>
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PSTC NWG Membership

• Abbott – Eric Blomme, Yi Yang
• Amgen – Dina Andrews
• Astra-Zeneca – Scott Adler ¹, Mark Pinches, Jenny McKay, Matthew Wagnor
• Boehringer-Ingelheim – Jonathan Phillips ¹, Jaromir Mikl
• Bristol-Myers Squibb – Denise Bounous
• ClinXus – William Baer, Gary Neidert
• Daiichi-Sankyo – Martins Adeyemo, Takashi Yamoto, Toshimasa Jindo, Shinya Sehata
• GSK – Daniela Ennulat
• EMA – Jean-Marc Vidal
• FDA – Peter Goering, Rodney Rouse
• Critical Path Institute – Elizabeth Walker, Phil Rossi, Eric Thompson, Eslie Dennis, Amanda Baker, Cassandra Mtine

• J&J – Eric McDuffie, Monisha Sonee
• Lilly – Diane Hamlin
• Merck – Eddie Gu, Dan Holder, Frank Sistare, Sean Trioth
• Mitsubishi Tanabe – Naohisa Tsutsui
• Novartis – Philip Bentley, Peter McArdle, Franck Meyer, Etsuko Usui
• Pfizer – Dominique Brees, Stefan Sultana
• Roche – Rabih Slim
• Sanofi-Aventis – Valerie Guilpin, Jean-Charles Gautier

• Academic consultants under CDA’s
  – Harvard: Joseph Bonventre
  – UAB: David Warnock
  – UCSD: Ravi Mehta

¹Co-Chair
“Data Shown” Acknowledgements

**Merck:**
- David Gerhold
- Zoltan Erdos
- Sean Troth
- Dan Holder
- Josef Ozer
- Warren Glaab
- Wendy Bailey
- Hong Jin
- Alema Galijatovic-Idrizbegovic
- Sanja Altman-Hamamdzic
- Tom Forest
- Raj Muniappa
- Hima Patel
- Holly Clouse
- Janet Kerr
- Carolanne Beare
- Tom Skopek
- Frank Sistare
- Doug Thudium
- Yan Yu
- Katerina Vlasakova

**Novartis:**
- Frank Dieterle
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- Andre Cordier
- Elias Perentes
- Olivier Grenet
- Peter Grass
- Daniel Wahl
- Andreas Mahl
- Robert Daniel Roth
- Frank Städtler
- Michael Kammüller
- Peter End
- François Legay
- Pablo Verdes
- Pierre Moulin
- Serrafino Pantano
- Salah-Dine Chibout
- Jacky Vonderscher

**Harvard:**
- Joseph Bonventre
- Vishal Vaidya

**Special thanks to:**
- Meso Scale Discovery
- Biotrin
- Rules Based Medicine
- CIT
- Biolytix
BACKUPS
• Early liver injury as detected by sensitive aminotransferase elevations does not often and only quite rarely progresses to serious damage and functional loss, and therefore are very poor predictors - early detection of injury does not identify the great majority of animals or people who can adapt to and become tolerant of a drug. 

BMs of kidney injury may or may not be predictive of serious damage to come – discuss individual patient basis vs population basis – tracking sCr w injury BM’s.

• Like the liver, the kidney also has capacity to adapt, change, and regenerate quickly


Liver damage mode of action capability? Promise of mRNA, miRNA.

The need for relevant animal models

• Animal sCr data vs injury data - the clinical qualification challenge

• Efavarinz is both a great kidney and liver story
Criteria adapted from Altar, et al, and Bradford-Hill for defining the strength of evidence to support the qualification of a new safety biomarker

- Availability of a sufficiently validated analytical assay
- Support for biological plausibility of a biomarker's association with organ injury
- Understanding of the molecular mechanism of the biomarker response
- Strength of association demonstrating linkage of the biomarker change to pathology outcome and improved performance relative to currently accepted biomarkers
- Consistency of response across mechanistically diverse and relevant kidney toxicants; and across sexes, strains, and species
- Presence of both a dose-response and temporal relationship relating the magnitude of the biomarker response to severity of injury, and the onset and recovery of injury to correlative and timely changes in the biomarker
- Appropriate specificity of the biomarker to not respond to agents which injure other organs but do not injure the kidney, or which activate physiological processes within the kidney without tissue injury
Nonclinical Progressive Qualification Objectives

• Overall approach is to further characterize performance of BMs using histopathology as gold standard and compare against BUN & sCr as current conventional and routine accessible BMs

• Six claim-oriented “progressive qualification” objectives:
  1. Establish additional novel biomarkers’ “added value” and/or “outperformance” of BUN/Serum Creatinine in acute renal injuries
  2. Establish evidence of renal injury biomarkers as leading indicators of early structural injury – assessment of prodromal character
  3. Establish biomarker performance for monitoring reversibility of drug-induced tubular and glomerular injuries
  4. Collect evidence of specific regional sensitivity of BMs (e.g., PT, DT, Loop of Henle, Collecting Duct, interstitium, pre-renal, etc.) and histologic process association (e.g., degeneration, regeneration, dilatation, etc.) to monitor drug-induced renal injuries
  5. Collect further evidence of general performance specificity
  6. Collect evidence of performance value in later onset/longer duration study designs
The FNIH Biomarker Consortium is led by an Executive Committee with senior-level participation from NIH, FDA, industry and other sectors.

**Chairman**
Charles Sanders, FNIH

**NIH**
Thomas Insel, NIMH
John Niederhuber, NCI
Lawrence Tabak, NIDCR

**FDA**
ShaAvhree Buckman, *Office of Translational Science*
Janet Woodcock, *Director of CDER*

**Public Member**
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