NEDMDG Summer Symposium

Burlington, MA

June13, 2016

Qualification of New Translational Safety Biomarkers for Drug Development

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Merck & Co., Inc.

On behalf of the C-Path Predictive Safety Testing Consortium
SKMWG, NWG and the FNIH Biomarkers Consortium KSP Team

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Presentation Outline

- The Need for New Translational Safety Biomarkers
- Criteria for Qualification of New Toxicity Biomarkers
- Consortium Approach to Biomarker Qualification
- New Translational Kidney Safety Biomarkers
- New Translational Skeletal Muscle Safety Biomarkers
- Summary

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The Need for New Translational Safety Biomarkers

- Drug-induced tissue injury can be a cause of compound failure in drug development, prevent clinical translation of these development compounds, and may result in the withdrawal of pharmaceuticals from the market
- Techniques used to diagnose and monitor certain tissue injuries in animals and humans have significant limitations, including lack of specificity and/or sensitivity
- Novel sets of accessible biomarkers of tissue injury have been identified, assays developed, and added value demonstrated
- Qualification of these novel biomarkers is in progress as part of the Critical Path Institute's Predictive Safety Testing Consortium (PSTC), seeking regulatory endorsement for their utility pre-clinically and in clinical settings

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Considerations for Novel Translational Safety Biomarker Qualification

- **Qualification** is the evidentiary process linking a biomarker with biological processes and clinical end points
- **Qualification** is also agreement that the evidence gathered is sufficient for a specific "context of use"

- Availability of *sufficiently validated analytical assay*
- *Biological understanding* and relevance to toxicity
- Understanding of *mechanism of response*
- Biomarker response that *reflects pathology* and demonstrates *improved performance* relative to conventional biomarkers
- *Consistent response* across mechanistically different compounds, and response similar across sex, strain, and species
- Presence of a *dose response and temporal relationship* to the magnitude of response
- *Specificity of response* to toxicity – understanding the response to toxicities in other tissues, or to pharmacologic effects without toxicity in the target organ

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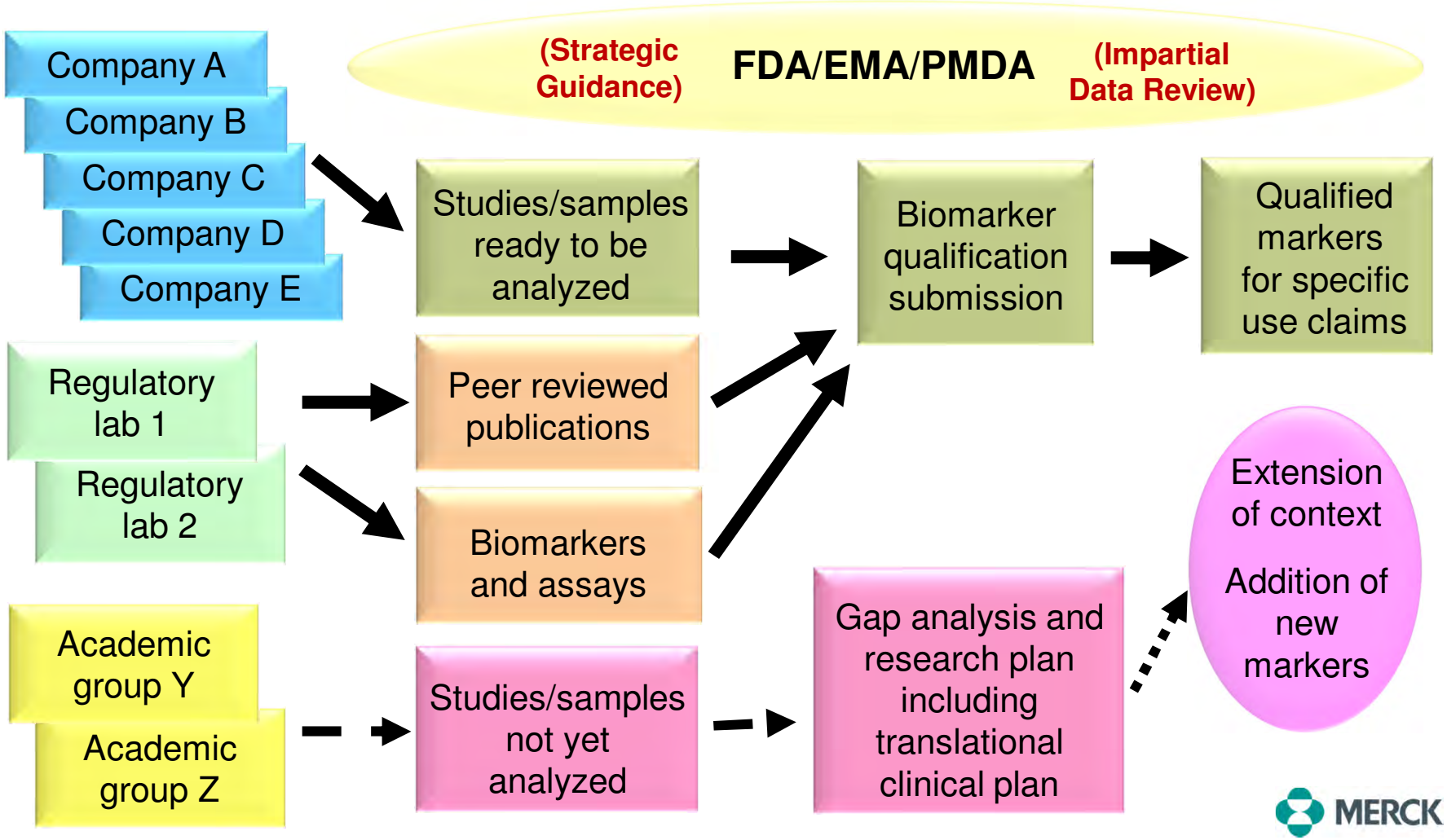
Qualification Process Through C-Path's Predictive Safety Testing Consortium (PSTC)

Goal: To gain industry and regulatory agreement to the reliability of novel safety biomarkers for nonclinical and clinical drug development applications



New Translational Kidney Safety Biomarkers

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New Qualified Translational Kidney Safety Biomarkers Could Enable Continued Development of Drugs Suspected of Human Irrelevant Renal Toxicities Observed in Animals

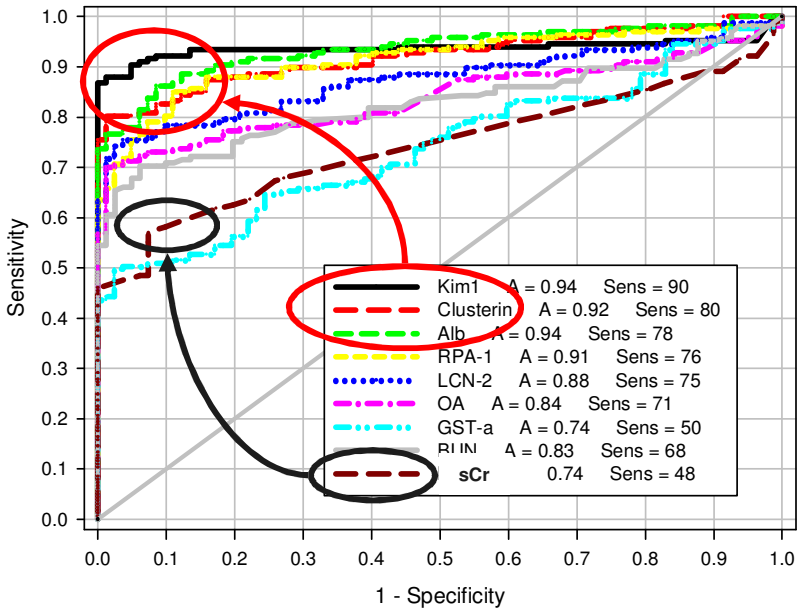
Problem: Histopathological lesions observed in animal studies at doses and times with no measureable changes in sCr. **Is this a single species effect and a human irrelevant mechanism?** Inability to confidently monitor patients triggers candidate attrition and significantly delays timelines for patients with significant medical needs.

2008 CPath PSTC Consortium Drives Regulatory Qualification

Following assessment, the EMA, FDA, PMDA concluded

- 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

- New kidney safety biomarkers outperform serum creatinine and BUN in rats
  - FDA, EMA, PMDA Qualification



Nature Biotech. May 2010; 10 manuscripts.

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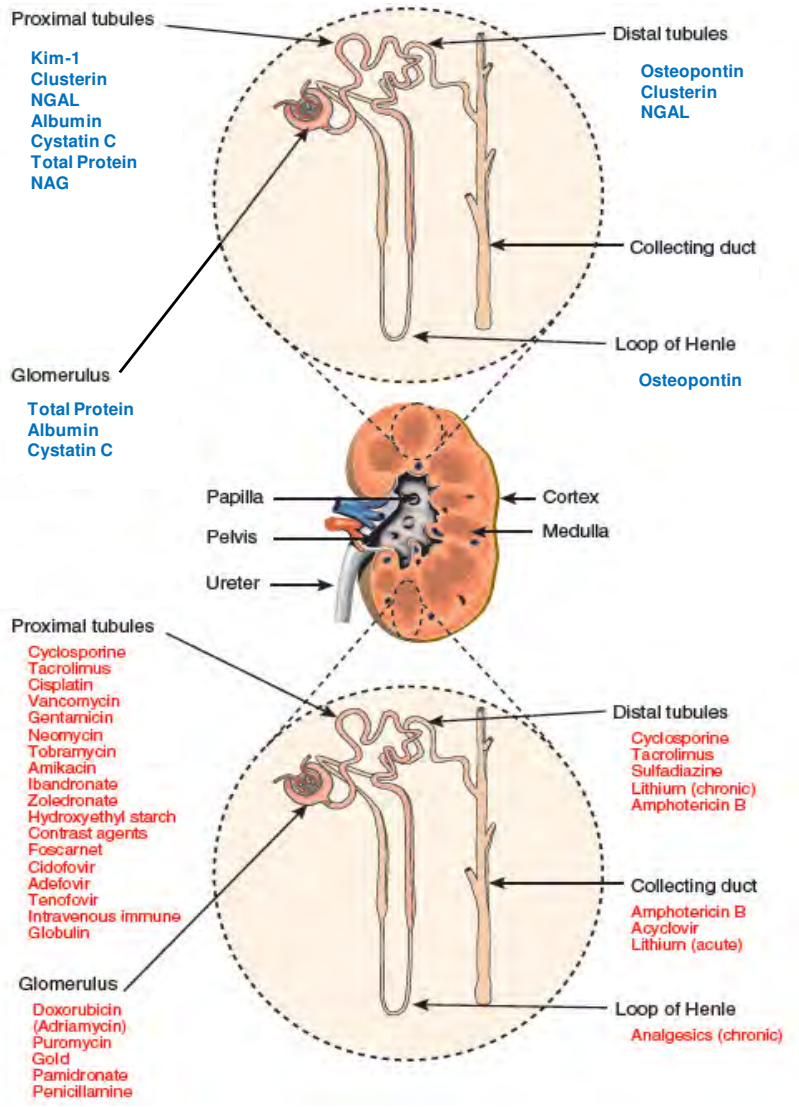
Promising Accessible Biomarkers of Acute Renal Damage or Dysfunction to Complement BUN and Serum Creatinine

Functional Biomarkers	Proposed Functional Interpretations
Albumin	Small quantities filtered by glomerulus and efficiently reabsorbed by tubular epithelium
Cystatin C	Normally highly filtered but glomerular or tubular damage yields protein overload that inhibits tubular reabsorption from lumen
Total Urinary Protein	Functional marker of glomerular filter integrity or tubular dysfunction
Injury Response Markers	**Proposed Structural Interpretations**
Clusterin	Necrotic tissue sequestration; and regenerative repair response present in many renal cell types
Kim-1	Tubular epithelium dedifferentiation and regenerative repair response
NGAL (Lipocalin 2)	Also filtered and reabsorbed; distal tubule inflammation and to sequester iron, limit damage
Osteopontin	Expressed in TAL and DCT, may limit oxidative stress and ischemia, and assist regeneration
Leakage Markers	
NAG	Brush-border enzyme released when damage occurs to tubular epithelium



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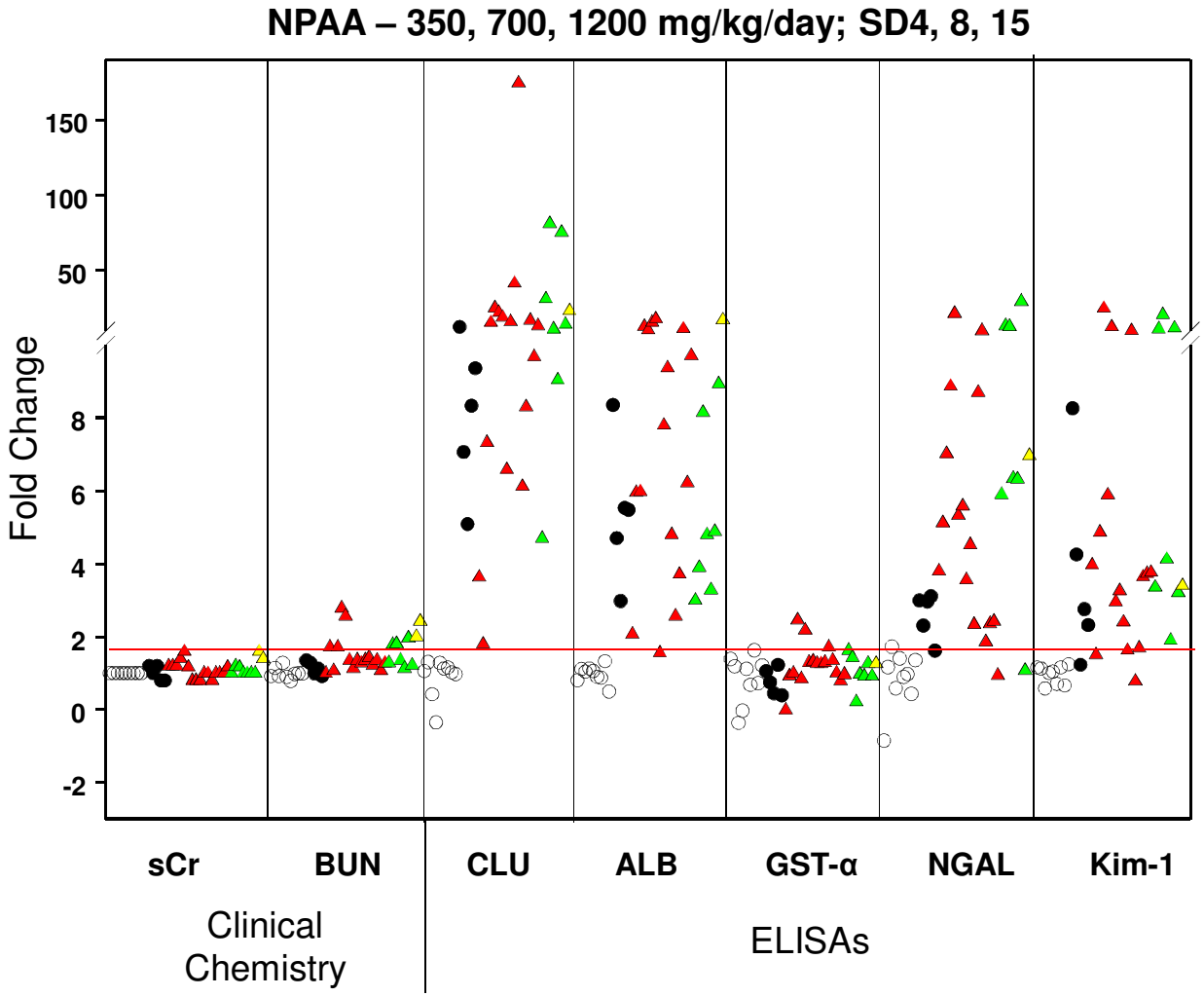
Example: Pre-Clinical Kidney Biomarker Performance in a 2-week NPAA Rat Toxicity Study



Histopath

- Control grade 0
- Treated grade 0
- Treated grade 1
- Treated grade 2
- Treated grade 3

Urinary biomarkers are more sensitive than BUN and sCr in diagnosing kidney toxicity

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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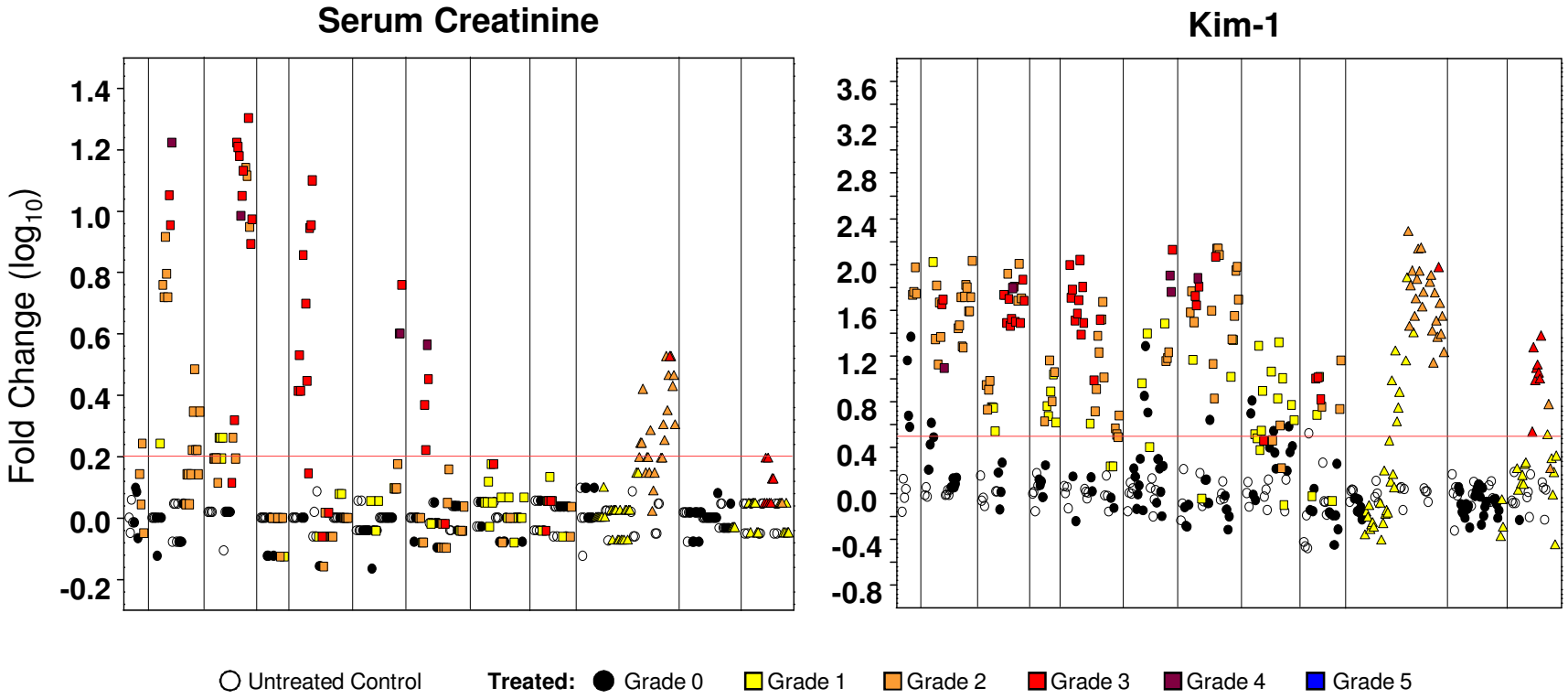
Kidney Biomarker Performance in 5 to 15 Day Rat Studies: Serum Creatinine vs Kim-1



Studies for both markers in order as follows:
bacitracin, carbapenem A, cisplatin, cyclosporin, D-serine, gentamicin, HCB, NPAA, propyleneimine, doxorubicin, puromycin, Thy1.1

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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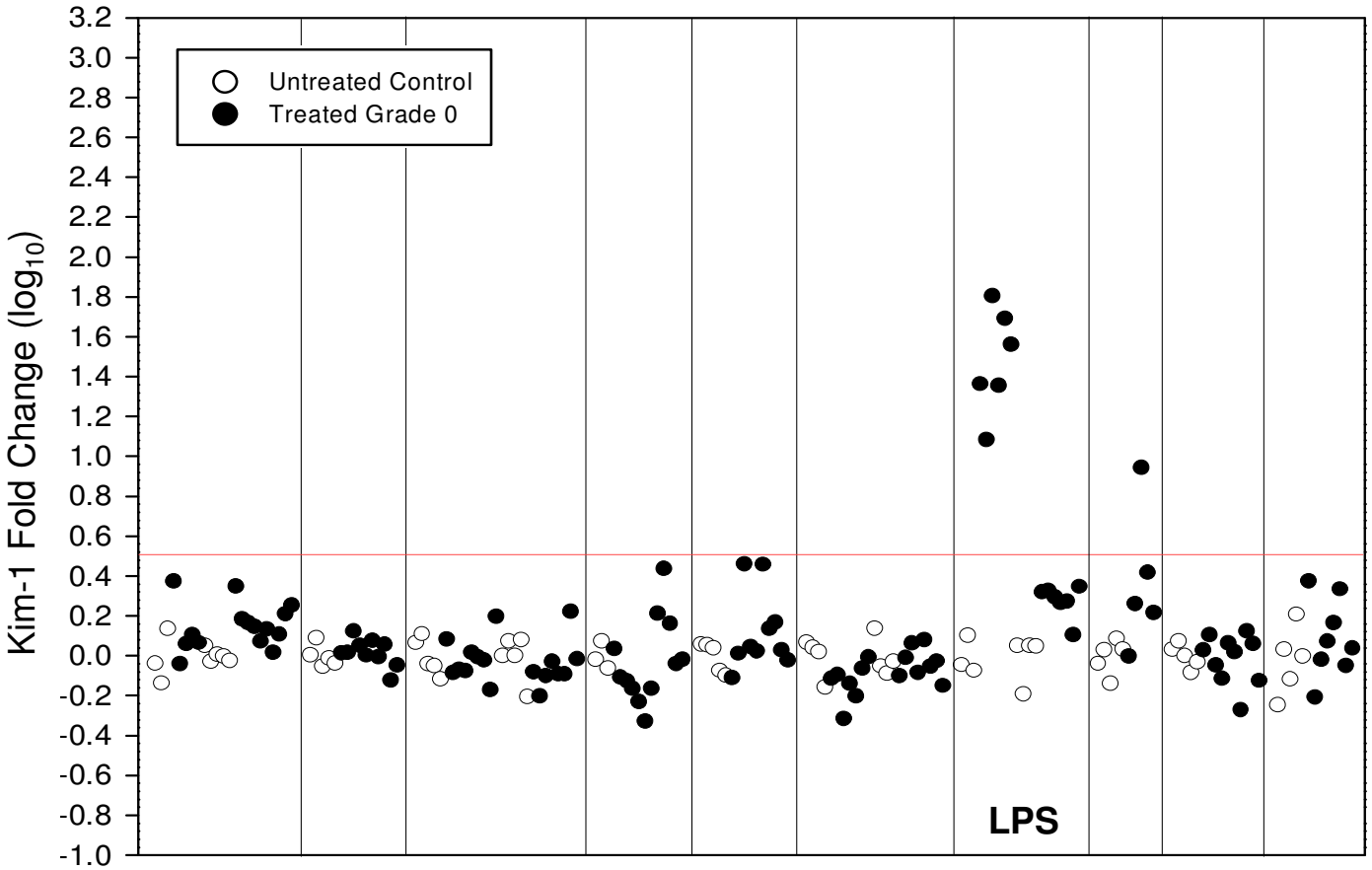
Data Set Example: Kim-1 Across 10 Specificity Studies in Rat



Studies in order as follows:

ethynyl estradiol, APAP, allylamine, bromobenzene, Merck A, Merck B, LPS, minoxidil, norethindrone, TMPD

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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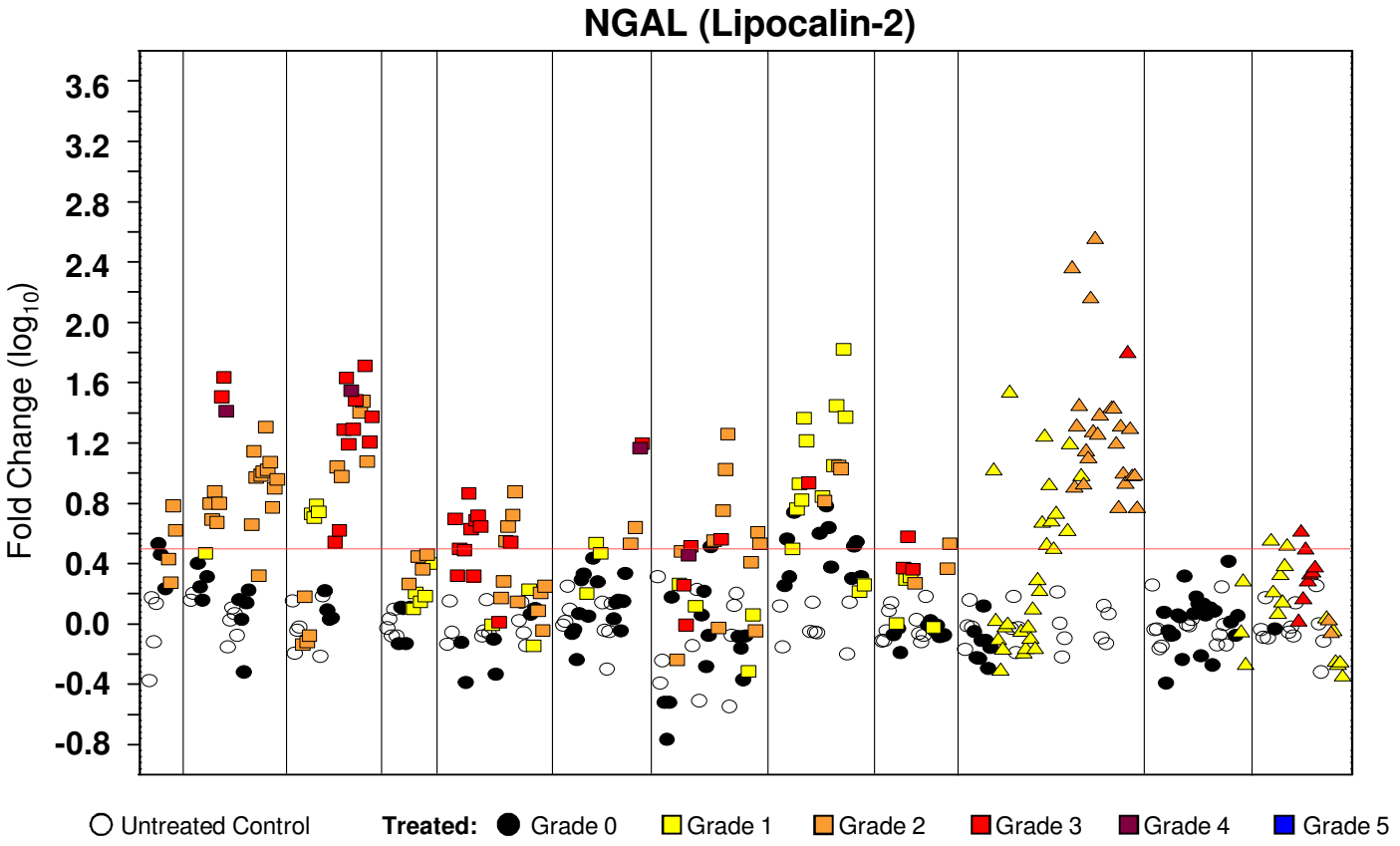
Data Set Example:
NGAL Across 12 Rat Kidney Toxicity Studies



In order as follows:
bacitracin, carbapenem A, cisplatin, cyclosporin, D-serine, gentamicin, HCB, NPAA, propyleneimine, doxorubicin, puromycin, Thy1.1

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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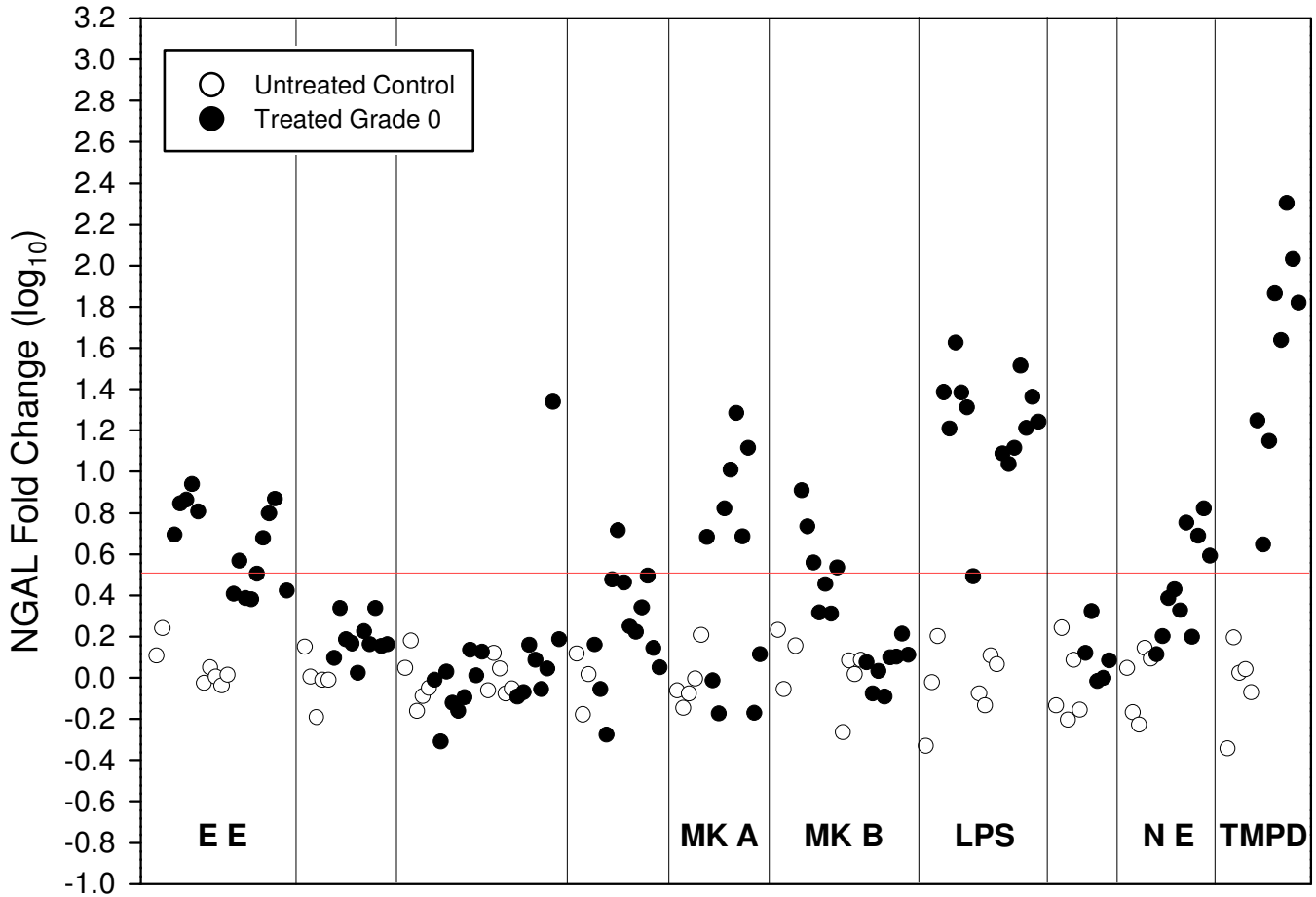
Data Set Example:
N-Gal Results Across 10 Specificity Studies



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Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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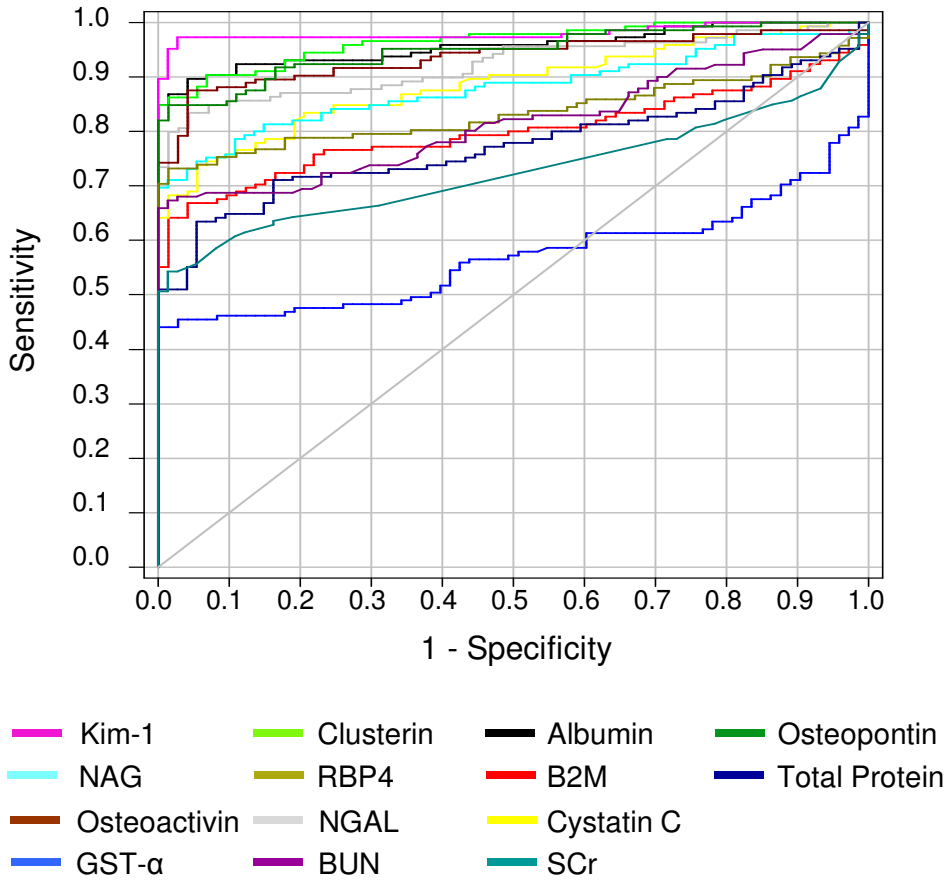
Summary Tubular Studies in Rat: 9 Sensitivity Studies

Tubular Sensitivity Studies Exclusion Model



	Sensitivity Studies – Exclusion Model		
Biomarker	**AUC**	**Sens***	**Fold Cut Off**
Kim-1	**0.98**	**97**	1.5
Clusterin	**0.96**	**86**	1.8
Albumin	**0.96**	**90**	1.8
Osteopontin	**0.95**	**85**	1.7
Osteoactivin	**0.94**	**88**	1.9
NGAL	**0.92**	**83**	1.6
Cystatin C	0.89	69	1.6
NAG	0.88	73	1.4
RBP4	0.84	73	1.8
B2M	0.80	67	1.6
Total Protein	0.77	55	1.8
GST-α	0.57	45	1.8
BUN	0.81	68	1.2
sCr	***0.73***	***56***	1.1

*Sensitivity values at 95% specificity.

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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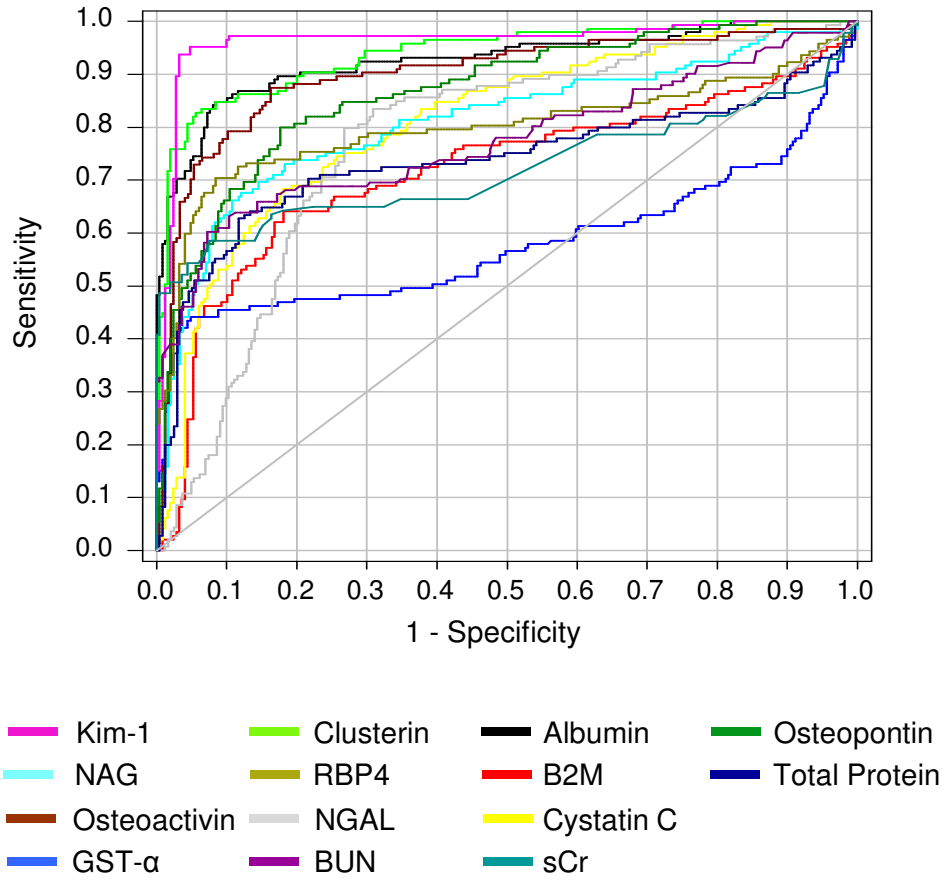
Summary Tubular Studies in Rat: 9 Sensitivity and 10 Specificity Studies

Tubular Sensitivity and Specificity Studies Exclusion Model



Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

	Sensitivity and Specificity Studies – Exclusion Model		
Biomarker	**AUC**	**Sens***	**Fold Cut Off**
Kim-1	**0.96**	**94**	2.4
Clusterin	**0.93**	**80**	3.7
Albumin	**0.92**	**73**	5.2
Osteopontin	0.86	52	7.2
Osteoactivin	0.90	66	4.5
NGAL	0.76	10	20
Cystatin C	0.80	37	5.9
NAG	0.81	47	2.5
RBP4	0.79	61	3.5
B2M	0.71	24	11
Total Protein	0.74	50	2.0
GST-α	0.58	44	2.2
BUN	0.77	46	1.7
sCr	***0.72***	***54***	1.1

*Sensitivity values at 95% specificity.

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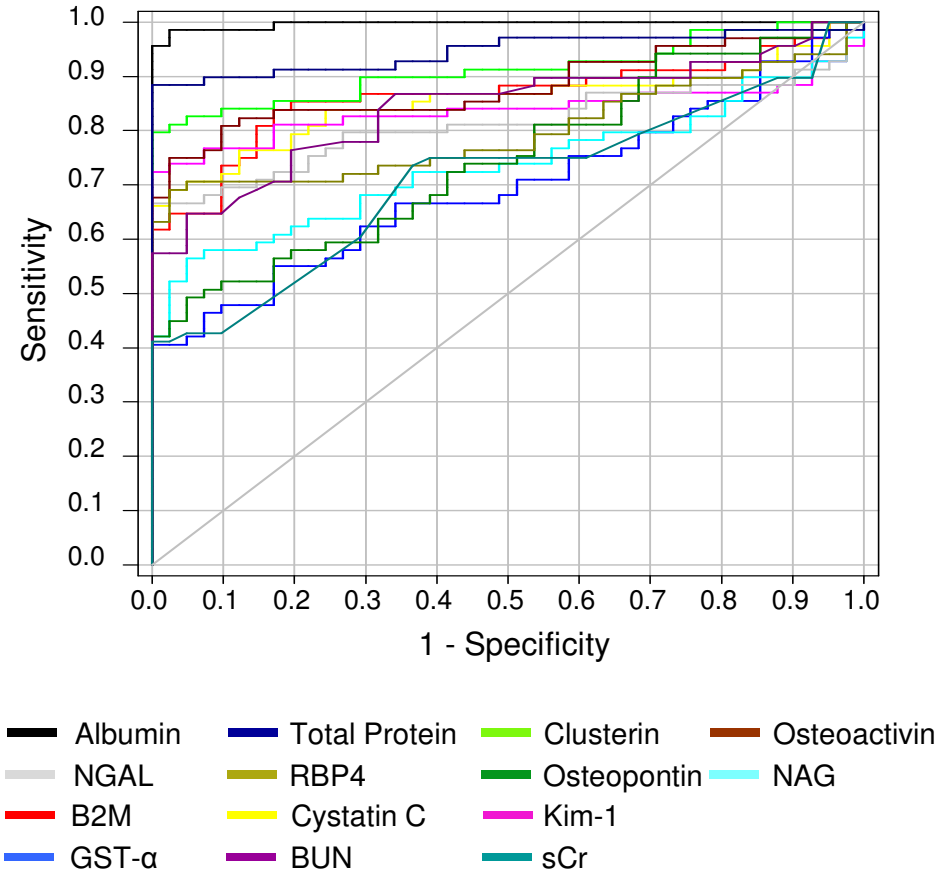
Summary Glomerular Studies in Rat: 3 Sensitivity and 10 Specificity Studies

Glomerular Sensitivity and Specificity Studies Exclusion Model



	Sensitivity and Specificity Studies – Exclusion Model		
Biomarker	**AUC**	**Sens***	**Fold Cut Off**
Albumin	**0.99**	**93**	6.8
Total Protein	**0.92**	**80**	2.1
Clusterin	0.87	64	4.5
Osteoactivin	0.84	62	4.3
B2M	0.78	43	16
Cystatin C	0.79	62	5.9
Kim-1	0.80	62	2.5
NGAL	0.67	12	21
RBP4	0.74	53	3.9
Osteopontin	0.58	12	7.6
NAG	0.64	30	2.7
GST-α	0.68	12	2.4
BUN	0.76	28	1.8
sCr	***0.69***	***43***	***1.2***

*Sensitivity values at 95% specificity.

Vlasakova K, et al. *Tox Sci.* 2014;138:3-20.

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Summary – Preclinical Qualification in Rat

- Initial preclinical qualification through PSTC of 7 kidney toxicity biomarkers (Kim-1, Clusterin, Albumin, Total Protein, Cystatin C, B2 microglobulin, and TFF3) completed on limited rat study set and regulatory endorsement received
- Qualification extended to 12 urinary biomarkers in 22 rat studies including both kidney toxicants and compounds with toxicities observed in organs other than kidney
- Kim-1, clusterin, and albumin showed the highest overall sensitivity for detecting drug-induced renal tubular injury, while albumin exceeded all other markers in detecting drug-induced glomerular injury
- All biomarkers demonstrated some lack of specificity, most notably NGAL and osteopontin, illustrating the need for caution when interpreting urinary biomarker increases in samples when organ toxicity is unknown

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Clinical Kidney Biomarker Qualification Project through the FNIH and PSTC

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Anticipated Results of FNIH BC and PSTC Kidney Clinical BMx Qualification Project (Collaboration of Consortia)

Qualify a new set of biomarkers that outperform sCr and BUN for monitoring response to a potential mild reversible kidney tubule injury in early clinical drug development

- 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 potential acute renal tubule injury with compounds dosed to relevant clinical exposures in Phase 1 or 2 clinical drug trials in subjects with normal renal function
- Provide practical thresholds of changes in these biomarkers that signify agreement to a mild reversible injury response (that might be used as stopping criteria to halt or modify dosing)
- This project will complement the ongoing clinical work supported by the European-based Innovative Medicines Initiative SAFE-T Consortium

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Data for Clinical Learning Phase

- Normal healthy volunteer cohort
  - N = 80, balanced on gender and age (~40/40, 20-39 years and 40-69 years)
  - Longitudinal sample collections over 3 weeks
- Cisplatin-treated mesothelioma patient cohort
  - N = 58 patients treated with surgical resection and 250 mg/m^2 intraoperative intrathoracic cisplatin (3% <40 years; 80% males; 62% ≥stage 2 CKD at baseline)
  - Longitudinal sample collections over 6 days

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Learning Phase Data Summary: 8 Selected Urinary Biomarkers Show Improved Sensitivity Over sCr to Identify Patients Exposed to Cisplatin

	Mesothelioma Patients: Number/N (%) >T_{SS}*		Normal Healthy Volunteers: % >T_{SS}
Biomarker	**Patients With Medically Relevant Increases in sCr**	**Patients Without Medically Relevant Increases in sCr**	**Normal eGFR No Cisplatin (N = 80)**
Clusterin	19/20 (95.0%)	**22/30 (73.3%)**	1.3%
Osteopontin	20/20 (100.0%)	**30/31 (96.8%)**	5.1%
Microalbumin	20/20 (100.0%)	**30/30 (100.0%)**	2.5%
Total Protein	20/20 (100.0%)	**30/30 (100.0%)**	3.8%
NAG	20/20 (100.0%)	**27/30 (90.0%)**	0%
Kim-1	20/20 (100.0%)	**30/30 (100.0%)**	1.3%
Cystatin-C	19/20 (95%)	**22/30 (73.3%)**	5.1%
NGAL	19/20 (95%)	**24/30 (80.0%)**	4.1%

*T_{SS} = statistically significant threshold.

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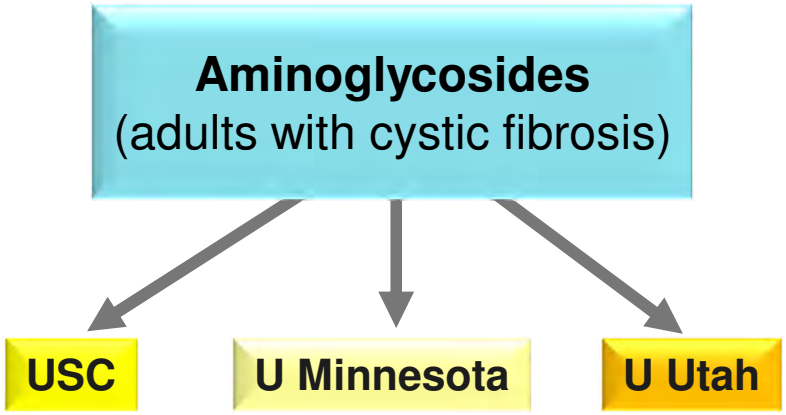
Two Prospective Clinical Trials Enrolling

100 patients/50 controls, blood/urine

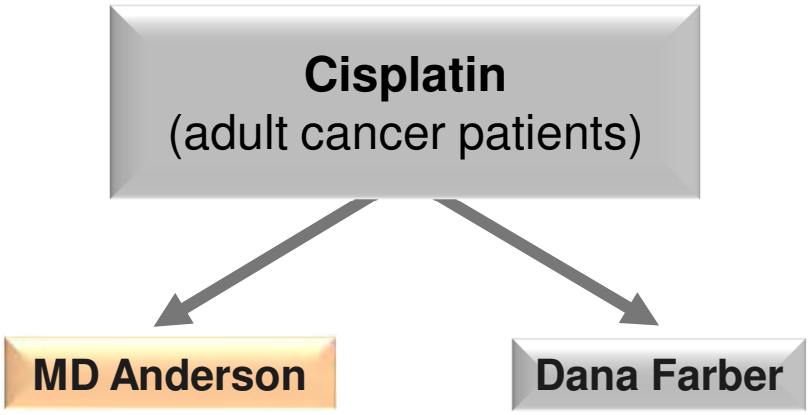


Patients (n=100)
Adult CF patients, acute pulmonary infection treated with IV tobramycin

Controls
Adult CF patients (n=25), acute pulmonary infection treated with IV fluoroquinolone;
Adult CF patients (n=25), no pulmonary infection, no treatment

(Current enrollment ~30%)

100 patients/50 controls, blood/urine



Patients (n=100)
Patients with head and neck squamous cell carcinoma, and other cancers treated with cisplatin as single agent or part of chemotx cocktail

Controls (n=50)
Cancer patients receiving non-cisplatin chemoTx treatment, or radiation Tx

(Current enrollment ~75%)

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Summary: "Learn and Confirm" Strategy

- Preclinical data with cisplatin, aminoglycoside, other renal toxicants demonstrates superiority of numerous biomarkers over sCr for monitoring renal tubular injury (using microscopic histopathology as gold standard)
- Clinical learning phase
  - Prospective healthy volunteer study
  - Archived samples from cisplatin study
  - Range of biomarkers investigated ➔ selected 8 for confirmatory phase
  - Established thresholds of selected biomarkers
- Clinical confirmatory phase
  - Aminoglycoside study in cystic fibrosis patients
  - Cisplatin study in cancer patients
- Submission of data supporting clinical qualification

Challenges of Clinical Qualification of Translational Safety Renal Biomarkers

- Resource commitments necessary for conducting proactive clinical trials to support clinical qualification of novel translational safety renal biomarkers in a clinical setting
  - Significant cost of conducting clinical trials
  - Limited number of compounds can be assessed (prioritization and selection process)
- The time commitment relative to the preclinical qualification presents a major hurdle
- The lack of a clinical “gold standard” (histopathology anchor) to assess performance of novel safety biomarkers
- Conducting clinical research through a committee of scientists uncertain access to funding sources

Summary: New Translational Kidney Safety Biomarkers

- Preclinical data with renal toxicants demonstrates superiority of numerous biomarkers over sCr for monitoring renal tubular and glomerular injury (using microscopic histopathology as gold standard)
- Preclinical qualification data provides the supporting evidence to advance regulatory acceptance for clinical applications of new “fit for purpose” renal safety biomarkers
- Current efforts focused on qualifying these new kidney biomarkers for use in early clinical trials to monitor response to a potential mild reversible kidney tubule injury

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New Translational Skeletal Muscle Safety Biomarkers

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Statement of Need for New Skeletal Muscle (SKM) Biomarkers

Nonclinical Species

- Serum aspartate aminotransferase (AST) and creatine kinase (CK) activity lack sensitivity and/or specificity (e.g. AST increases in liver injury)

Humans

- AST typically not considered a SKM injury biomarker
- Serum CK activity is helpful but not sufficient – small increases in CK activity are difficult to interpret
- Clinical symptoms are ambiguous – differences in terminology and the subjective nature of self-reporting
- SKM wasting prevalent in many chronic conditions – drug-induced SKM toxicity highly undesirable with treatment

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Overview of Skeletal Muscle Injury Biomarkers

Four protein biomarkers to monitor skeletal muscle injury (defined as myocyte degeneration/necrosis):

1. Skeletal troponin I (sTnI) – component of myofilaments. Expression restricted to skeletal muscle.
2. Myosin light chain 3 (Myl3) – component of myofilaments. Abundant in skeletal muscle; also expressed in cardiac muscle.
3. Fatty-acid binding protein 3 (Fabp3) – cytosolic lipid transport protein. Abundant in skeletal muscle; also expressed in cardiac muscle.
4. Creatine kinase muscle type (Ckm) – cytosolic metabolic protein. CK-MM homodimer highly abundant in skeletal muscle.

*Also evaluated additional biomarkers that did not meet performance criteria (urinary myoglobin, serum/plasma parvalbumin)

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Results in Support of a "Letter of Support" (LOS)

- Objective: Determine if SKM biomarkers outperform CK and/or AST.
- Data set: 34 rat toxicity studies contributed by member companies.
  - 18 sensitivity studies covering a broad range of drug-induced skeletal muscle injury mechanisms.
  - 16 specificity studies including liver(5), kidney(5), liver & kidney (2), gastrointestinal(3) and vascular(1) drug-induced injury.
- Supporting activities:
  - Development of histopathology lexicon.
  - Cross-site validation of assay performance at 4 member labs.
  - One chronic canine study.
  - Study in aged (1 year old) rats.

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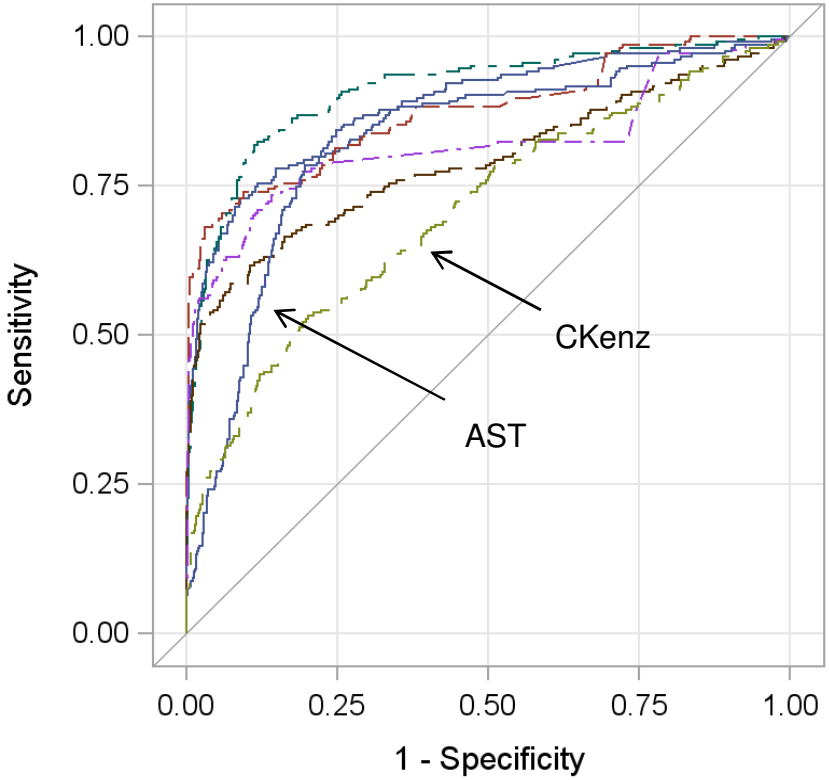
ROC Curves Show SKM Biomarkers Outperform CK and AST

34 rat toxicity studies contributed by member companies.

- 18 sensitivity studies covering a broad range of skeletal muscle injury mechanisms.
- 16 specificity studies: liver(5), kidney(5), liver & kidney (2), GI (3), vascular injury (1)



	ROC Curve line	AUC
Ckm		0.9093
Fabp3		0.8814
Myl3		0.8729
AST		0.8232
sTnI		0.8193
CK		0.7057

	Prob AUC > AUC_{AST}	Prob AUC > AUC_{CK}	Sensitivity at 95% Specificity
Ckm	<0.0001	<0.0001	65%
Fabp3	<0.0001	<0.0001	64%
Myl3	<0.0116	<0.0001	69%
AST	NA	<0.0001	26%
sTnI	0.8628	0.0001	59%
CK	<0.001	NA	28%

- Ckm, FABP3 and Myl3 outperform CK and AST
- sTnI outperforms CK and similar performance to AST

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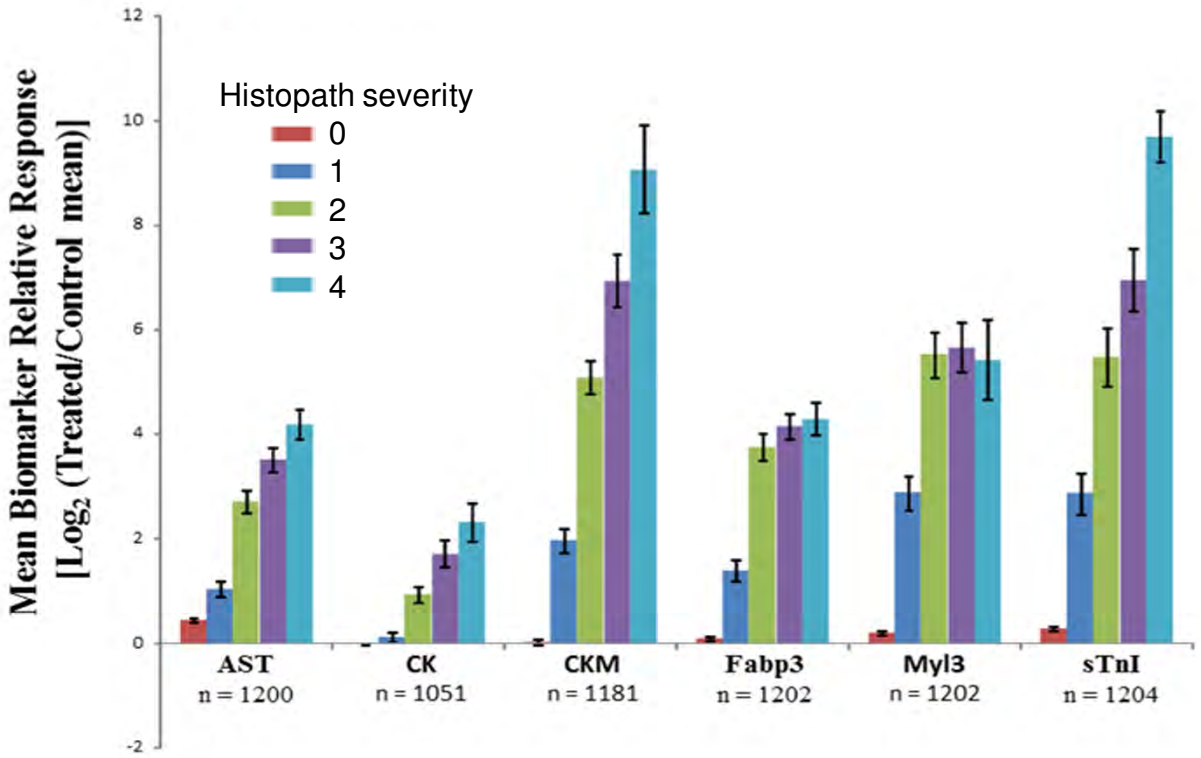
SKM Biomarkers Correlate with Muscle Histopathology Severity Score



- Severity of SKM degeneration/necrosis scored by histopathology
- With increased injury severity, SKM biomarkers increase
- Fold change much greater for most novel SKM biomarkers

Spearman correlation:

	Ckm	Fabp3	Myl3	sTnI	AST	CK
Correlation coefficient	0.5372	0.5178	0.4847	0.4567	0.4697	0.3217
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

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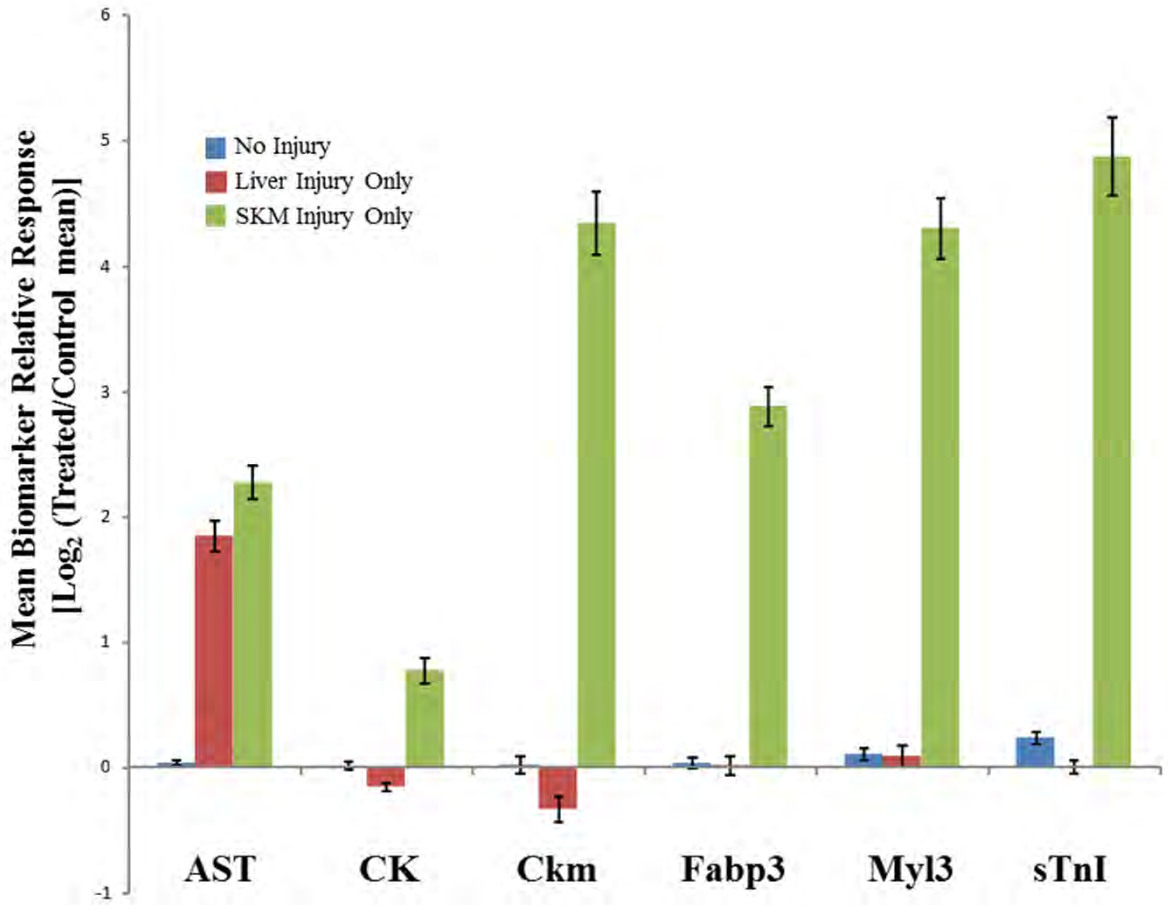
Improved Specificity over AST when Liver Injury is Present



- Identified animals with no injury, only liver or only SKM injury by histopathology
- AST shows marked response over vehicle control with both liver and SKM injury
- SKM biomarkers and CK show little to no response to liver injury

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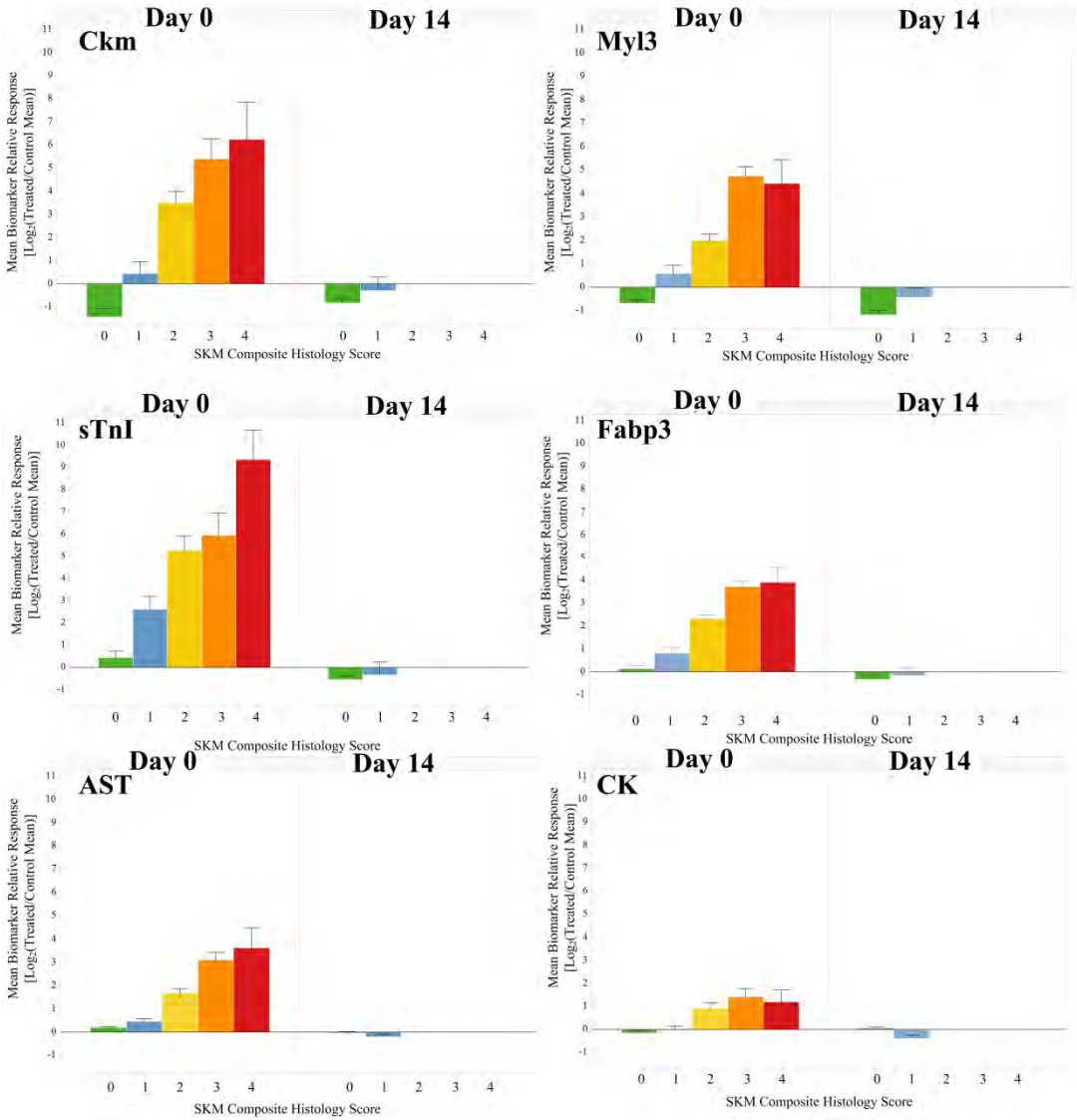
SKM Biomarker Levels Correlate with Resolution of SKM Necrosis/Degeneration



- Six studies had 14 day recovery arms
- Analyzed biomarker response relative to vehicle control on day 0 and 14 after dosing by histopathology score
- All biomarkers returned to vehicle control levels by day 14, along with resolution of histopathology

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Overall Results

Biomarker	Improves overall sensitivity and specificity when used with AST and CK	Greater sensitivity than AST and CK at 95% specificity	Greater sensitivity and specificity than AST and CK	Improves diagnostic certainty when combined with AST and CK	Levels in blood correspond to severity of SKM injury
Ckm	✓	✓	✓	✓	✓
Fabp3	✓	✓	✓	✓	✓
Myl3	✓	✓	✓	✓	✓
sTnI	✓	✓	CK only	✓	✓

Letter of Support received from the FDA and EMA in 2015 for the use of sTnI, Myl3, Fabp3 and Ckm as biomarkers of SKM degeneration/necrosis in preclinical development, as well as encouraging their use in early clinical trials

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Clinical Skeletal Muscle Biomarker Qualification Project

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SKM Biomarker – Clinical Translation Strategy

- Select SKM biomarkers for clinical qualification based on biomarker data from rat tox studies and published data for humans COMPLETED
- Validate assays for these biomarkers in human matrices COMPLETED
- Establish ranges for various patient populations IN PROGRESS
  - Healthy volunteers
  - Individuals with conditions of interest
  - Effect of exercise
- Investigate effect of various medications on SKM biomarkers: Statins and Fibrates IDENTIFYING PATIENT SAMPLES
- Interactions between key variables: exercise, medications, disease state FUTURE EFFORT

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Summary: New Translational Safety Biomarkers

- Preclinical data with renal and skeletal muscle toxicants demonstrates superiority of numerous biomarkers over existing biomarkers (using microscopic histopathology as gold standard)
- Preclinical qualification data provides the supporting evidence to advance regulatory acceptance for clinical applications of new “fit for purpose” safety biomarkers
- Current efforts focused on qualifying these new biomarkers for use in early clinical trials to monitor response to a potential mild reversible kidney tubule and skeletal muscle injury

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Acknowledgements

CPath PSTC SKMWG and NWG
FNIH BC Kidney Safety Biomarker Project Teams

Abbvie: William Bracken

Amgen: Wayne Tsuji

Bristol-Myers Squibb: Debra Wescott

AstraZeneca: Scott Adler

C-Path: Amanda Baker, Martin Cisneroz, Eslie Dennis, Nicholas King, Gary Lundstrom, John-Michael Sauer, Elizabeth Walker

FDA: Melanie Blank, Aliza Thompson

FNIH: Steve Hoffman, Jessica Ratay, Maria Vassileva

Brigham & Women's Hospital: Sushrut Waikar, Robert Haddad

Genentech: Tanja Zabka

Johnson & Johnson: James Lenhard

Lilly: Greg Hall, Richard Higgs, Michael Pritt, James Voelker, David Watson

Merck: Warren Glaab, Robin Mogg, Irene Nunes, Frank Sistare, Michael Tanen, Michael Topper,

NIH/NIDDK: Paul Kimmel

PSTC: Stefan Sultana (Novartis) Jaromir Mikl (Boehringer-Ingelheim) Steven Piccoli (BMS)

Pfizer: Peter Burch, Gary Friedman, Richard Giovanelli, Richard Goldstein

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