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

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

- **Company A**
- **Company B**
- **Company C**
- **Company D**
- **Company E**

- **Regulatory lab 1**
- **Regulatory lab 2**

- **Academic group Y**
- **Academic group Z**

- **(Strategic Guidance)**
- **FDA/EMA/PMDA** (Impartial Data Review)

- **Studies/samples ready to be analyzed**
- **Peer reviewed publications**
- **Biomarkers and assays**
- **Studies/samples not yet analyzed**

- **Gap analysis and research plan including translational clinical plan**

- **Qualified markers for specific use claims**

- **Extension of context**
- **Addition of new markers**

**MERCK**
New Translational Kidney Safety Biomarkers
**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.](image)
### Functional Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</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>Cystatin C</td>
<td>Normally highly filtered but glomerular or tubular damage yields protein overload that inhibits tubular reabsorption from lumen</td>
</tr>
<tr>
<td>Total Urinary Protein</td>
<td>Functional marker of glomerular filter integrity or tubular dysfunction</td>
</tr>
</tbody>
</table>

### Injury Response Markers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Proposed Structural Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clusterin</td>
<td>Necrotic tissue sequestration; and regenerative repair response present in many renal cell types</td>
</tr>
<tr>
<td>Kim-1</td>
<td>Tubular epithelium dedifferentiation and regenerative repair response</td>
</tr>
<tr>
<td>NGAL (Lipocalin 2)</td>
<td>Also filtered and reabsorbed; distal tubule inflammation and to sequester iron, limit damage</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Expressed in TAL and DCT, may limit oxidative stress and ischemia, and assist regeneration</td>
</tr>
</tbody>
</table>

### Leakage Markers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Proposed Functional Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAG</td>
<td>Brush-border enzyme released when damage occurs to tubular epithelium</td>
</tr>
</tbody>
</table>
Example: Pre-Clinical Kidney Biomarker Performance in a 2-week NPAA Rat Toxicity Study

NPAA – 350, 700, 1200 mg/kg/day; SD4, 8, 15

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

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

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

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

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

Summary Tubular Studies in Rat: 9 Sensitivity Studies

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

*Sensitivity values at 95% specificity.

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


Tubular Sensitivity and Specificity Studies
Exclusion Model

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

*Sensitivity values at 95% specificity.
Summary Glomerular Studies in Rat: 3 Sensitivity and 10 Specificity Studies

Glomerular Sensitivity and Specificity Studies
Exclusion Model

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC</th>
<th>Sens*</th>
<th>Fold Cut Off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.99</td>
<td>93</td>
<td>6.8</td>
</tr>
<tr>
<td>Total Protein</td>
<td>0.92</td>
<td>80</td>
<td>2.1</td>
</tr>
<tr>
<td>Clusterin</td>
<td>0.87</td>
<td>64</td>
<td>4.5</td>
</tr>
<tr>
<td>Osteoactivin</td>
<td>0.84</td>
<td>62</td>
<td>4.3</td>
</tr>
<tr>
<td>B2M</td>
<td>0.78</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.79</td>
<td>62</td>
<td>5.9</td>
</tr>
<tr>
<td>Kim-1</td>
<td>0.80</td>
<td>62</td>
<td>2.5</td>
</tr>
<tr>
<td>NGAL</td>
<td>0.67</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>RBP4</td>
<td>0.74</td>
<td>53</td>
<td>3.9</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>0.58</td>
<td>12</td>
<td>7.6</td>
</tr>
<tr>
<td>NAG</td>
<td>0.64</td>
<td>30</td>
<td>2.7</td>
</tr>
<tr>
<td>GST-α</td>
<td>0.68</td>
<td>12</td>
<td>2.4</td>
</tr>
<tr>
<td>BUN</td>
<td>0.76</td>
<td>28</td>
<td>1.8</td>
</tr>
<tr>
<td>sCr</td>
<td>0.69</td>
<td>43</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Sensitivity values at 95% specificity.

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

- 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
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/m² intraoperative intrathoracic cisplatin
    (3% <40 years; 80% males; 62% ≥stage 2 CKD at baseline)
  - Longitudinal sample collections over 6 days
Learning Phase Data Summary: 8 Selected Urinary Biomarkers Show Improved Sensitivity Over sCr to Identify Patients Exposed to Cisplatin

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Patients With Medically Relevant Increases in sCr</th>
<th>Patients Without Medically Relevant Increases in sCr</th>
<th>Normal eGFR No Cisplatin (N = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clusterin</td>
<td>19/20 (95.0%)</td>
<td>22/30 (73.3%)</td>
<td>1.3%</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>20/20 (100.0%)</td>
<td>30/31 (96.8%)</td>
<td>5.1%</td>
</tr>
<tr>
<td>Microalbumin</td>
<td>20/20 (100.0%)</td>
<td>30/30 (100.0%)</td>
<td>2.5%</td>
</tr>
<tr>
<td>Total Protein</td>
<td>20/20 (100.0%)</td>
<td>30/30 (100.0%)</td>
<td>3.8%</td>
</tr>
<tr>
<td>NAG</td>
<td>20/20 (100.0%)</td>
<td>27/30 (90.0%)</td>
<td>0%</td>
</tr>
<tr>
<td>Kim-1</td>
<td>20/20 (100.0%)</td>
<td>30/30 (100.0%)</td>
<td>1.3%</td>
</tr>
<tr>
<td>Cystatin-C</td>
<td>19/20 (95%)</td>
<td>22/30 (73.3%)</td>
<td>5.1%</td>
</tr>
<tr>
<td>NGAL</td>
<td>19/20 (95%)</td>
<td>24/30 (80.0%)</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

*T_{ss} = statistically significant threshold.
Two Prospective Clinical Trials Enrolling

100 patients/50 controls, blood/urine

**Aminoglycosides**
(adults with cystic fibrosis)

**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

**USC**
**U Minnesota**
**U Utah**

100 patients/50 controls, blood/urine

**Cisplatin**
(adult cancer patients)

**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

**MD Anderson**
**Dana Farber**

(Current enrollment ~30%)

(Current enrollment ~75%)
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.
New Translational Skeletal Muscle Safety 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
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)*
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.
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)

<table>
<thead>
<tr>
<th></th>
<th>ROC Curve line</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ckm</td>
<td>—</td>
<td>0.9093</td>
</tr>
<tr>
<td>Fabp3</td>
<td>—</td>
<td>0.8814</td>
</tr>
<tr>
<td>Myl3</td>
<td>—</td>
<td>0.8729</td>
</tr>
<tr>
<td>AST</td>
<td>—</td>
<td>0.8232</td>
</tr>
<tr>
<td>sTnl</td>
<td>—</td>
<td>0.8193</td>
</tr>
<tr>
<td>CK</td>
<td>—</td>
<td>0.7057</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Prob AUC &gt; AUCAST</th>
<th>Prob AUC &gt; AUCCK</th>
<th>Sensitivity at 95% Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ckm</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>65%</td>
</tr>
<tr>
<td>Fabp3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>64%</td>
</tr>
<tr>
<td>Myl3</td>
<td>&lt;0.0116</td>
<td>&lt;0.0001</td>
<td>69%</td>
</tr>
<tr>
<td>AST</td>
<td>NA</td>
<td>&lt;0.0001</td>
<td>26%</td>
</tr>
<tr>
<td>sTnl</td>
<td>0.8628</td>
<td>0.0001</td>
<td>59%</td>
</tr>
<tr>
<td>CK</td>
<td>&lt;0.001</td>
<td>NA</td>
<td>28%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Ckm</th>
<th>Fabp3</th>
<th>Myl3</th>
<th>sTnI</th>
<th>AST</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.5372</td>
<td>0.5178</td>
<td>0.4847</td>
<td>0.4567</td>
<td>0.4697</td>
<td>0.3217</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
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
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
### Overall Results

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Improves overall sensitivity and specificity when used with AST and CK</th>
<th>Greater sensitivity than AST and CK at 95% specificity</th>
<th>Greater sensitivity and specificity than AST and CK</th>
<th>Improves diagnostic certainty when combined with AST and CK</th>
<th>Levels in blood correspond to severity of SKM injury</th>
</tr>
</thead>
<tbody>
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<td>✔</td>
<td>CK only</td>
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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.
Clinical Skeletal Muscle Biomarker Qualification Project
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
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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