### ABSTRACT

Drug-induced kidney injury (DIKI) is not an uncommon adverse event in drug development. A remaining challenge is the late identification of Acute Kidney Injury due to the current standards (i.e. serum creatinine (Cr) and blood urea nitrogen (BUN)) which may not be significantly changed until 2/3 of the kidney function has already been lost.

Over the last three years there has been great progress with preclinical qualification processes for kidney biomarkers. The PSC and ILSI HES have both had rat kidney biomarkers qualified by both the FDA and EMA. These landmark qualifications mean that drug companies may now use certain novel preclinical markers for end decision making within their drug discovery process.

The principal objective of this new project is to collect and generate sufficient clinical data from a number of candidate kidney biomarkers, that will provide convincing evidence for the health authorities to endorse these biomarkers for the detection and monitoring of drug induced kidney injuries in specific clinical situations. To address this, a European-based partnership called the SAFE-T Consortium was formed from 20 participants from the pharmaceutical industry, small-medium enterprises, academic institutions and clinical units.

### SAFE-T OBJECTIVES

**Primary objectives**
1. To develop and qualify biomarkers that detect DIKI earlier in man than the currently used standards.
2. To gain scientific acceptance and regulatory endorsement for the use of these biomarkers in defined clinical contexts.

**Secondary objectives**
- a. Assessment of sensitivity & specificity of biomarkers for injury in specific compartments of the kidney (e.g. glomeruli, tubules, collecting ducts)
- b. Characterization of the effect on biomarkers of acute and chronic non-renal organ diseases
- c. Determination of inter- and intra-individual variability of the candidate biomarkers in healthy individuals and patients with chronic kidney diseases and the associated normal ranges.
- d. Evaluation of the potential of biomarkers to track the onset and monitor the resolution of kidney injury.
- e. Exploration of the clinical utility when these biomarkers are used to trigger intervention.

### RESULTS

**CANDIDATE KIDNEY BIOMARKERS UNDER ASSESSMENT**

<table>
<thead>
<tr>
<th>Candidate Name</th>
<th>Qualifier</th>
<th>Platform</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIM-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGAL (urine &amp; serum)</td>
<td></td>
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<td></td>
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<tr>
<td>Clustersin</td>
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<td></td>
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<tr>
<td>TFF3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Timp-1</td>
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<td></td>
<td></td>
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<tr>
<td>CTGF</td>
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<td></td>
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<tr>
<td>NLR-18</td>
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<td></td>
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<tr>
<td>MCP-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcibindin D2B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clusterin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomarker Panel</td>
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<tr>
<td>Changes of protein levels in urine and blood reflect different pathological processes in different compartments of the kidney.</td>
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</tbody>
</table>

**ASSAY VALIDATION PROCESS**

SAFE-T is using commercially available assays for the candidate biomarkers selected where available. Through a series of low and high bar validation processes the markers have been assessed for their suitability for entry into the clinical sample assessment. For some of the markers that are available on multiple technologies (ELISA, Luminex, Mesoscale, etc.) the consortium has undertaken dual technology assessment.

**Low Bar Validation**

Low bar refers to a basic manufacturer’s specifications technical validation of the assay.

- Precision - Inter and intra assay variation of standard curve and samples.
- Sensitivity - Lower limit of detection determination.
- Recovery - Spiking urine matrix with known concentration of analyte.
- Linearity - dilution series of a high sample.

**High Bar Validation**

High bar refers to a full technical validation of the assay. More detailed experiments will be carried out such as, any interference factors which may exist, reference range generation (age, gender etc) and lot to lot variability. This will then lead to the establishment of a final QC SOP and then the further study of the assay according to GLO standards.

**SUCCESSFUL BIOMARKER SELECTION PROCESS**

**Exploratory Phase**
- The biomarkers will be assessed in healthy volunteer cohorts. Markers with unacceptable high variability in these samples will be dropped.
- Next the candidates will be assessed for their response to drug induced kidney injury. Those markers with poor discrimination in these cohorts will be dropped.
- A number of non-renal disease cohorts will be collected. These samples should not elicit a significant response in the candidate biomarkers.
- Finally, a cohort of CKD samples will be collected. Candidate patterns in CKD will be very important to understand.

### SAFE-T APPOINTMENT: THE KIDNEY BIOMARKER QUALIFICATION WORKFLOW

- **Problem Statement**
  - Being more effective than serum creatinine and BUN for AKI diagnosis is easy. Evidence based proof to submit to the regulators is more of a challenge! How does one compare new biomarkers with a Clinical Evidence standard (Cr)?

  - There is an urgent need for the qualification of biomarkers that detect and monitor drug induced kidney injury.

- **SAFE-T APPROACH: THE KIDNEY BIOMARKER QUALIFICATION WORKFLOW**

  - **Current Standards**
    - Serum Creatinine
    - Albumin
    - Clusterin
    - Biomarker Panel

  - **Markers being investigated**
    - Total Protein
    - Biomarker Panel
  
- **Candidates**

  - KIM-1
  - NGAL (urine & serum)
  - Clustersin
  - TFF3
  - Osteopontin
  - Timp-1
  - CTGF
  - NLR-18
  - MCP-1
  - Calcibindin D2B

- **Status**

  - Under validation
  - Validated

### HOW TO ASSESS THE PERFORMANCE OF NOVEL BIOMARKERS

SAFE-T have adopted the AKIN criteria for the definition of acute kidney injury and its three stages. This classification is dependent on serum creatinine which has been described as a weak standard for AKI.

**How do you assess a novel biomarker against Scr without histology?**

SAFE-T has proposed an approach to novel biomarker assessment (which has been discussed with the regulatory authorities (FDA & EMA). The approach involves the establishment of adjudication committees to determine the relevance of increases of biomarkers in the absence of increases of serum creatinine and whether these are true positive or false positive signals (i.e. post-hoc judgment of the patient assignments [control vs nephrotoxicant] on the basis of blinded review of)

1. **Standard clinical parameters**
2. **Biomarker profiles**
3. **Biomarker profiles and clinical parameters**

**There is much work to complete but this study presents the opportunity to assess (on a large scale) the true value of novel biomarkers in assessing drug induced kidney injury.**