INTRODUCTION

The aim of the safety biomarker program at Pacific Biomarkers (PacBio) is to assess the occurrence of organ injury due to specific toxic effects of drugs in development that could prevent their approval. This will avert injury to patients that would have been treated by drugs whose toxicity would fail to be detected by current methods and will further reduce healthcare costs by early recognition that a drug is not safe as the result of drug-induced toxic effects. The latter is expected to speed up drug development by several years. Current costs related to the development of a drug from discovery to the final approval are estimated to be about $1 billion. Drug-induced toxicity accounts for 30% of all drug failures prior to reaching the market, and the sooner this toxicity is discovered, the sooner further costly studies could be stopped. The PacBio safety biomarker program has the potential to diagnose toxic drug effects that currently are being missed in clinical trials.

A strategic goal for PacBio is to provide pharmaceutical and biotech companies with services for testing robust novel biomarkers that have undergone thorough analytical validation and clinical qualification to diagnose early organ injury. This work is taking place in response to FDA-initiated recommendations for streamlining and improving drug development outcomes as outlined in the Critical Path Initiative through guidance from the Predictive Safety Testing Consortium (PSTC) and the Health and Environment Sciences Institute (HESI). During the past 20 years, PacBio has acquired extensive experience in biomarker standardization as a member of the Cholesterol Reference Method Laboratory Network and work in other areas of diagnostic test improvement. The anticipated qualification of organ injury biomarkers will also demand development of stringent standardization procedures (e.g., consistency in test accuracy, precision, and sensitivity).

AIM

The first initiative in PacBio’s safety biomarker program is to target biomarkers with the clinical, preanalytical and analytical qualities that current literature and experimental evidence indicate have the greatest potential for early detection of treatment (drug)-induced acute kidney injury (AKI). This is being followed by a similar evaluation of markers associated with treatment-induced injury for liver and heart. PacBio is currently collaborating with the research and development leaders in this field to characterize the analytical and clinical performance of these novel renal biomarkers.

PacBio has already characterized the analytical performance of a range of AKI biomarkers (see Table 1). PacBio suggests utilizing the biomarkers that have been recommended by the various consortia and key opinion leaders in the field, are analytically valid and appear to be stable until measured. Biomarkers meeting these criteria are marked by an asterisk (Table 1) and cover both specific and global renal injury.

APPROACH

The PacBio approach to analyzing AKI biomarkers begins with validate assays for each biomarker using rigorous protocols for singlicate determination, with the expectation of generating the most robust data possible. During this process, PacBio has often been required to modify existing assay methods. Thus, the best possible analytical performance, i.e., superior precision, accuracy, sensitivity and specificity, has been the goal. Quality analytical performance has been sought to ensure that during the current period of the clinical qualification of these biomarkers, the data are not tainted by poor analytical and preanalytical performance. This approach incorporates clinical considerations while establishing a consensus for the best AKI biomarkers. The eventual consensus on five to seven biomarkers will lead to their use with unreserved confidence.
Thorough evaluation and years of experience utilizing the validated AKI biomarker assays has provided PacBio with the insight to pinpoint which AKI biomarkers will prove best for monitoring kidney injury. With this knowledge, PacBio is now moving forward with investigations of multiplex procedures for AKI biomarkers. Multiplexing will reduce cost and improve testing turnaround time. While the analytical performance may deteriorate significantly during multiplexing for some biomarkers, PacBio has given high priority to establishing excellent performance for the individual biomarkers before attempting to multiplex them. This provides assay performance expectations for use in the multiplex assay evaluation.

Issues surrounding the best methods of collecting and storing urine used for detection of AKI in human subjects are being addressed at Pacific Biomarkers. Our observations indicate that some of the candidate biomarkers are susceptible to loss associated with non-specific binding from the small aliquots of urine prepared for storage. This effect could reasonably be expected given the relatively low protein concentration in most urine samples, and it is reflected in the variability of the loss of biomarker from one urine collection to another. Different urine compositions, even from the same subject, have variable protein concentrations; however, when high enough, the non-specific loss is less problematic. Of course, which urine collections will be affected cannot be predicted, but fortunately, the effect is easily overcome by addition of a small volume of a ‘carrier’ protein-containing preservative. The resulting dilution is accounted for by normalization of biomarker level using urine creatinine.

### CONCLUSIONS

PacBio is well positioned to undertake support for biomarker candidates for early AKI detection because:

- PacBio has a long-standing history of employing rigorous validation standards for esoteric biomarkers, and this expertise and commitment has been used to initiate, develop, and validate renal injury biomarker assays.

- PacBio has meticulously evaluated the analytical and preanalytical performances of all of the most promising biomarkers of AKI. This allows pharmaceutical and biotech companies involved in development of novel therapeutics to incorporate these biomarkers in their clinical trials with a clear set of performance expectations.

- PacBio has extensive expertise in dealing with preanalytical issues associated with biomarker testing, and has researched the effects of the usual handling procedures on urinary AKI biomarker recovery.

- PacBio provides general and study-specific recommendations for specimen collection and storage that are reasonable and that are based on in-house biomarker recovery data.

- PacBio is well suited to complete the transfer of the most clinically efficacious of these biomarker assays to multiplexed methods.

### PROXIMAL TUBULES

- KIM-1
- Clusterin
- NGAL
- GST-α
- β2-microglobulin
- NAG
- Osteopontin
- Cystatin C (urinary)
- Netrin-1
- RBP
- IL-1B
- HGF
- Cyr61
- NHE-3
- Exosomal fetuin-A
- Albumin

### DISTAL TUBULES

- Osteopontin
- Clusterin
- GST-α
- NGAL
- H-FABP
- Calbindin D28

### COLLECTING DUCT

- Calbindin D28

### LOOP OF HENLE

- Osteopontin
### Table 1: AKI Biomarker Clinical Utility and Methodology

<table>
<thead>
<tr>
<th>NAME</th>
<th>TYPE OF INJURY AND LOCATION</th>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Markers of Specific Renal Tissue Injury</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney Injury Molecule 1 (KIM-1)*1–3</td>
<td>Proximal tubule</td>
<td>EIA</td>
</tr>
<tr>
<td>Neutrophil Gelatinase-associated Lipocalin (NGAL)*2–7</td>
<td>Proximal tubular epithelium and lysosomal enzyme release</td>
<td>EIA</td>
</tr>
<tr>
<td>N-Acetyl-β-D Glucosaminidase (NAG)*7</td>
<td>Proximal tubule</td>
<td>Enzyme Activity</td>
</tr>
<tr>
<td>Interleukin 18 (IL-18)*7</td>
<td>Apoptotic processes in the tubule epithelium</td>
<td>ECLIA</td>
</tr>
<tr>
<td>Clusterin*8</td>
<td>Tubule epithelium</td>
<td>EIA</td>
</tr>
<tr>
<td>Osteopontin*9</td>
<td>Distal tubule</td>
<td>EIA</td>
</tr>
<tr>
<td><strong>Markers of Global Renal Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystatin C*2</td>
<td>Glomerular, in urine specific for tubule damage</td>
<td>Immunoturbidimetric</td>
</tr>
<tr>
<td>Albumin</td>
<td>Glomerular and tubular function</td>
<td>Immunoturbidimetric</td>
</tr>
<tr>
<td>Total Protein</td>
<td>Glomerular and tubular function</td>
<td>Turbidimetric</td>
</tr>
<tr>
<td>β₂-microglobulin</td>
<td>Glomerular and tubular function</td>
<td>Immunoturbidimetric</td>
</tr>
<tr>
<td><strong>Emerging Markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver-type Fatty Acid Binding Protein (L-FABP)*7</td>
<td>Ischemic injury if tubular epithelium</td>
<td>EIA</td>
</tr>
<tr>
<td>α₂-Microglobulin</td>
<td>Glomerular injury</td>
<td>Immunoturbidimetric</td>
</tr>
<tr>
<td>Osteoactivin</td>
<td>Proximal tubular epithelial injury</td>
<td>EIA</td>
</tr>
<tr>
<td>Retinol-binding Protein</td>
<td>Proximal tubular epithelial injury</td>
<td>EIA</td>
</tr>
<tr>
<td>α-Glutathione S-transferase (α-GST)*10</td>
<td>Proximal tubular epithelial injury</td>
<td>EIA</td>
</tr>
<tr>
<td>γ-Glutamyl Transferase (γ-GT)*10</td>
<td>Tubular epithelial injury</td>
<td>Enzyme Activity</td>
</tr>
<tr>
<td>π-Glutathione S-transferase (π-GST)</td>
<td>Distal tubular epithelial injury</td>
<td>EIA</td>
</tr>
<tr>
<td>Type IV Collagen</td>
<td>Glomerular injury</td>
<td>EIA</td>
</tr>
</tbody>
</table>

*PBI recommends the use of these key AKI biomarkers for the detection of specific and global renal injury.

**METHOD KEY**

- EIA – Enzyme-linked Immunosorbent Assay (ELISA)
- ECLIA – Electrochemiluminescence Immunoassay


