



# MYRIAD RBM™

## Rat KidneyMAP®

**A 12-Biomarker Multi-Analyte Profile (MAP)**

for Detecting Kidney Toxicity in Rat Preclinical Drug Studies.

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### Introduction

#### **Rat KidneyMAP® and Biomarker Qualification by the Predictive Safety Testing Consortium**

Drug induced toxicity is responsible for killing 30% of promising compounds from early preclinical studies and can negatively impact both clinical development programs and approved drugs. Upwards of 20% of all clinical cases of acute kidney injury (AKI) occur as a result of drug exposure.

To address the need for improved detection of drug-induced nephrotoxicity, Myriad RBM, in close collaboration with the Predictive Safety Testing Consortium (PSTC), created a specific biomarker panel called Rat KidneyMAP®. The Rat KidneyMAP was instrumental in the success of the PSTC's first set of studies, published in the May 2010 focus issue of Nature Biotechnology, that detail the regulatory (FDA and EMEA) qualification of kidney biomarkers for preclinical use in detecting drug-induced kidney injury [1-12]. Herein we review the key findings and implications of this milestone and why biomarker panels like the Rat KidneyMAP are becoming standard practice for preclinical toxicity studies.

## The Need for Better Kidney Toxicity Biomarkers

Previous standards to identify nephrotoxic kidney injury, namely serum creatinine (SCr) and blood urea nitrogen (BUN), have long been recognized as inadequate. In patients with a large renal reserve, significant injury may not be reflected by changes in SCr until the injury is well established. In rodent preclinical studies, substantial injury needs to occur before changes in already low SCr levels are detectable. In addition, changes in SCr may not specifically represent kidney injury but may reflect muscle breakdown instead. Similarly, change in BUN is not a reliable indicator of kidney injury, as many factors such as protein loading and volume status can affect BUN concentrations. While histopathology can give an accurate depiction of the extent and site of kidney injury, it may not detect the early signs of damage; nor is it an option in the clinical trial setting and only rarely in the acute or chronic care situation.

For these reasons, the need for earlier and more sensitive biomarkers of kidney injury cannot be understated. The demanding task of filtering and eliminating waste products along with regulating fluid and electrolyte balance makes the kidney particularly susceptible to toxic injury. Besides the high intratubular drug concentration that occurs during normal physiologic filtration, active uptake and countercurrent exchange can also lead to high intracellular levels of nephrotoxicants and their metabolites in the tubular epithelium.

## The Predictive Safety Testing Consortium and the Qualification of Renal Biomarkers

The Nephrotoxic Working Group (NWG) of the Predictive Safety Testing Consortium (PSTC) was established as a collaborative effort between industry, regulatory agencies and academic stakeholders with the aim of identifying and qualifying kidney safety biomarkers. The goal was to qualify biomarkers under the Voluntary eXploratory Data Submission (VXDS) process. These biomarkers could then be used by the US FDA and the European Medicines Agency (EMA) to make regulatory decisions and identify more accurate testing methods to advance pre-clinical as well as clinical safety testing. Candidate biomarkers needed to provide additional or complimentary information to traditional standards (e.g. SCr, BUN and histopathology) in order to be useful for non-clinical development studies to detect acute drug-induced kidney toxicity as well as improve medicinal chemistry, dosing, and protocol decision-making (**Table 1**). Eventual use in human clinical trials will be considered on a case-by-case basis.

In the first round of qualification studies, 23 candidate biomarkers were initially selected by the NWG for evaluation in rat models of drug induced kidney injury. Of these, 7 biomarkers (**Table 2**) were presented by the PSTC and qualified by the FDA and EMA after extensive characterization of diagnostic sensitivity (percent positives correctly identified) and specificity (percent negatives correctly identified) as determined by comparison to the histological scoring to assess the severity of injury. The biomarkers were compared to SCr and BUN in time-course rat studies that included dosing with a variety of well known nephrotoxicants. Together, these biomarkers allow for earlier and more accurate detection of drug-induced kidney injury, representing a significant advancement over previous standards. In addition, the PSTC clearly blazed a needed pathway for the regulatory qualification of safety biomarkers in the preclinical and clinical setting.

## Qualification Summary (see Ref 7)

Urinary Kim-1, Clusterin, and Albumin individually outperformed SCr and BUN in the detection of proximal tubule injury. Clusterin was also highly specific, with no false-positives in response to hepatotoxicants. Urinary Cystatin C,  $\beta$ 2-Microglobulin, and total urinary protein individually outperformed SCr and BUN in detecting glomerular injury or damage resulting in impairment of tubular reabsorption. Total protein had the highest specificity and Cystatin C and  $\beta$ 2-microglobulin were the most sensitive markers. By simultaneously monitoring both glomerular and tubular markers, one can tell the sequence of injury in situations where glomerular injury leads to subsequent tubular protein overload resulting in tubular injury. For this reason, some of the PSTC glomerular markers have been reported in the literature as tubular injury markers. Urinary TFF-3 did not outperform SCr and BUN, but the reduction in TFF-3 levels occurred before histological signs of damage. It was also noted that reduced levels of TFF-3 may represent a false-positive indication of injury and the assay is not widely available. However, when combined with albumin, TFF3 can provide complementary information to SCr and BUN.

The last research report in the series addressed two significant deficiencies arising from the first round of submissions by the PSTC [12]. First, urinary biomarker performance was assessed during recovery after reversible nephrotoxic injury. The biomarkers trended or returned to baseline

### Table 1: Desired characteristics of renal safety biomarkers

- Readily accessible in body fluids or tissues
- Early, sensitive, and dose-dependent response to injury
- Specific to site of injury (i.e. glomerular, tubular, etc)
- Demonstrates recovery from injury
- Similar response across species

### Table 2: Seven Kidney Biomarkers Qualified For Preclinical Use By The FDA and EMA

1. KIM-1
2. Albumin
3. Total Protein
4.  $\beta$ 2-microglobulin
5. Cystatin C
6. Clusterin
7. Trefoil Factor-3

levels during recovery, proving their utility in monitoring renal toxicity, repair, and function. The second deficiency addressed the evaluation of the candidate serum biomarker cystatin C, a renal functional marker that has previously been demonstrated to out-perform SCr as an indicator of glomerular filtration and kidney function [15]. Serum cystatin C was clearly superior to SCr and BUN in detecting a range of kidney lesions, including tubular injury, dilatation and regeneration, intratubular casts, glomerular alterations and interstitial fibrosis. While urinary biomarkers are ideally suited for assessing proximal and distal tubule damage, these studies demonstrate that a serum biomarker may be more appropriate for identifying more global forms of kidney damage. It also offers the advantage of having another testing option when urine is not available.

### The Value of Other Not Yet Qualified Kidney Biomarkers

The other biomarkers in Myriad RBM's Rat KidneyMAP have been noted in multiple studies as candidates for nephrotoxic injury and can add value in characterizing the nature of a nephrotoxic insult (Table 3). They can be divided into two general categories: urinary proteins with enzymatic activity and filtered low-molecular weight proteins [16]. Because enzymes are generally larger proteins that are not freely filtered, an increase in these urinary proteins indicates shedding from damaged tubular cells, demonstrating that these enzymes can be markers for tubular injury and in some cases localizing the damage to a particular segment of the nephron or even sub-cellular locations. Among these are the glutathione-S-transferases (GSTs), which are important not only in a physiological context but also as indicators for drug metabolism in the kidney [17]. Specifically, GST $\alpha$  is expressed in the proximal tubule while GST $\mu$  is expressed in the distal tubule [18, 19], and an increase in GST protein has been demonstrated to correlate with severity of kidney injury[20].

A host of filtered low molecular-weight proteins have also been identified as biomarkers of nephrotoxic injury. In addition to  $\beta$ 2-Microglobulin, Clusterin, Cystatin-C, and Kim-1; Calbindin, Epidermal Growth Factor (EGF), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Osteopontin (OPN), Tissue Inhibitor Metalloproteinase type-1 (TIMP-1) and Vascular Endothelial Growth Factor A (VEGF-A) are included in the Rat KidneyMAP (Table 3). Increased levels of most of these low molecular-weight proteins in urine are biomarkers of proximal tubule injury where loss of reabsorption generally indicates injury to tubular epithelium. The vast preponderance of primary drug-induced kidney injury (>90%) occurs in the glomerulus or proximal tubule; however, several of the other proteins just mentioned can be useful markers of other segments of the nephron (Table 3).

**Table 3: Rat KidneyMAP™ Biomarkers**

GLOMERULAR INJURY BIOMARKERS						
Biomarker	Response to drug-induced injury	Site of injury	Qualified for preclinical use	Qualified for clinical use*	Outperforms SCr, BUN	Refs
$\beta$ 2-microglobulin	↑ urinary concentration up to several hundred fold	Glomerular (podocytes/filtration barrier) or proximal tubules	✓	✓	✓	[8,13]
Cystatin-C	↑ urinary concentration; ↑ serum concentration	Glomerulus and proximal tubule	✓	✓	✓	[8, 11, 15]
TUBULAR INJURY BIOMARKERS						
Biomarker	Response to drug-induced injury	Site of injury	Qualified for preclinical use	Qualified for clinical use†	Outperforms SCr, BUN	Refs
Calbindin	↓ tissue expression (response to cisplatin); ↑ urine concentration	Distal tubule, collecting duct				[13, 22]
Clusterin	↑ gene and protein expression	Proximal and distal tubules	✓	Pending	✓	[8, 23, 24]
EGF	↓ urinary excretion and tissue expression	Distal tubule and collecting duct				[25]
GST- $\alpha$	↑ urinary excretion and tissue expression	Proximal tubule				[18, 20]
GST- $\mu$	↑ urinary excretion and tissue expression	Distal tubule				[19]
Kim-1	↑ expression in PT cells; ↑ extracellular domain in urine	Proximal tubule	✓	✓	✓	[11, 13, 14]
NGAL	↑ urinary excretion	Proximal and distal tubule				[11, 13, 26]
OPN	↑ urinary excretion	Proximal, distal tubule and collecting duct				[11, 13]
TIMP-1	↑ urinary excretion	Proximal and distal tubule				[27]
VEGF-A	↑ urinary excretion	Reno-protective responses				[28]

\* Qualified for clinical use refers to a case-by-case context, not general qualification.

## The way forward: Application of PSTC qualified biomarkers in drug development

The focus of the initial qualification process by the PSTC was to identify biomarkers that can out-perform SCr and BUN for monitoring kidney injury in test animals and potentially provide early clinical studies with greater sensitivity, specificity and earlier detection of hepatotoxicity. The next stage of biomarker qualification for clinical studies is ongoing, but in the meantime the utility of these biomarkers extends through many phases and applications of drug development and safety testing. For example, kidney injury biomarkers can expedite translational development from pre-clinical animal studies to early clinical testing. They can be integrated with a pharmacokinetic/pharmacodynamic (PK/PD) drug development strategy, serving as indicators of PD and contributing to more informed decisions regarding range and interval of dosing. In particular, these biomarkers may assist in identifying toxicities that may have previously occurred below detectable levels, thereby providing a more accurate determination of initial human trial dose and dosing titration. When considered along with PK/PD modeling, these biomarkers can also help establish go/no go criteria when bridging from pre-clinical to phase I clinical studies. Taken together, these factors can help identify poor drug candidates early and prevent costly late-stage failure.

### Conclusion

Developed and validated in conjunction with the PSTC Nephrotoxicity Working Group, Myriad RBM's Rat KidneyMAP® comprises a panel of renal injury biomarkers that is highly useful in the early identification and characterization of kidney toxicity. Myriad RBM's Rat and Human KidneyMAPs can provide valuable information throughout drug development; from lead optimization to pre-clinical and clinical protocol decision-making.

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