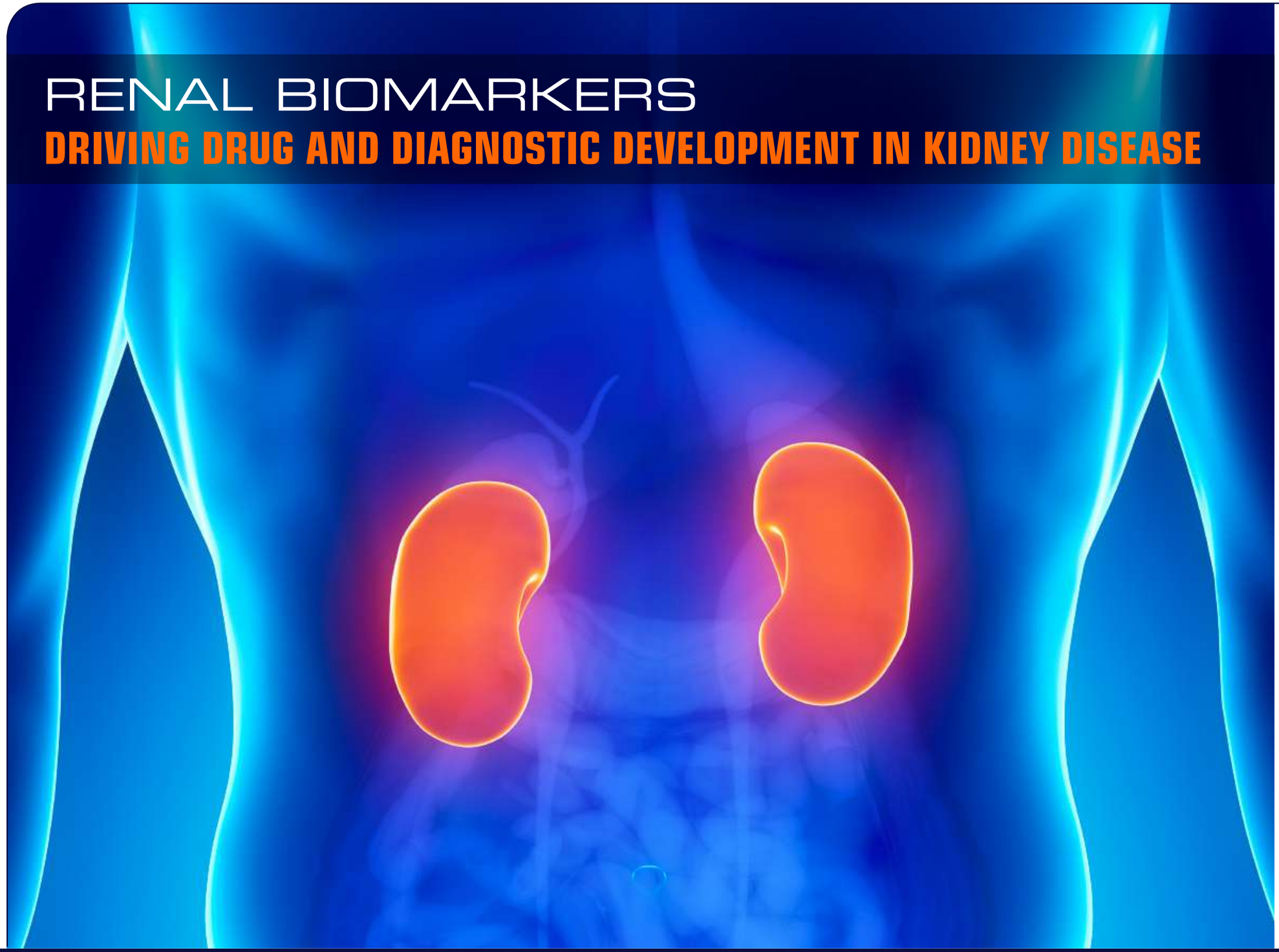
RENAL BIOMARKERS

DRIVING DRUG AND DIAGNOSTIC DEVELOPMENT IN KIDNEY DISEASE



The number of people with different forms of kidney disease, including nephropathies, drug-induced kidney toxicities and other renal disorders, is growing worldwide. Kidney disease, in whatever form it takes, has a significant effect on quality of life, and care for those who suffer from it puts a major burden on healthcare systems.

"When I began my career as a physician-scientist and a nephrologist, I saw that chronic kidney disease was a huge public health problem, and there were major gaps in our understanding of the disease," says Kumar Sharma, founder and chief scientific adviser at Clinical Metabolomics. The company, also known as ClinMet, uses its clinically based, targeted metabolomics platform to support drug discovery, development, and trials for kidney disease, diabetes, obesity and cardiovascular disease.

Numerous biomarkers, including serum and urine creatinine, blood urea nitrogen (BUN) and urine albumin, have been used in the diagnosis and monitoring of kidney disease for many years. However, these are most effective in established disease, and by the time they detect injury, the damage may already be done. Biomarkers that can pick up kidney problems in the very early stages could support drug development to treat earlier stage disease, as well as acting as an early warning system for drug-induced kidney toxicity before too much damage is done.

"We believe biomarkers have the potential to prevent significant cost to the healthcare system by catching a disease cascade early where preventive steps can be taken to prevent an unfavorable outcome," says Ralph McDade, strategic development officer of Myriad RBM, which provides protein biomarker products and services.

BY SUZANNE ELVIDGE

THE NEED FOR NEW RENAL BIOMARKERS

Kidney toxicity is a major issue in certain classes of drugs. The right biomarkers or combinations of biomarkers could spot the damage before it becomes irreversible, or predict which patients are most or least likely to experience side effects. Biomarkers also play a role in kidney disease, but there has been less work in this area.

"This may partly be because kidney disease is often seen as a result of other diseases, such as diabetes, obesity, or hypertension, and so the target is treating the causes rather than the outcomes. It may also be because there is a lack of understanding about the mechanism of the disease, so it's difficult to find and develop new drugs," says Ian Pike, COO at Proteome Sciences, a company focusing on protein and peptide biomarker solutions for pharmaceutical and diagnostics applications.

In both of these areas, renal biomarkers play a role at a number of different of stages:

- Identifying people at risk
- Spotting the damage early
- Monitoring development of damage
- Tracking efficacy of treatments
- Predicting outcomes:
  - The patients most (or least) likely to recover from an acute injury
  - The patients who need the most care
  - The patients who will benefit the most from treatment
- Acting as a companion diagnostic for marketed drugs.

The ideal biomarker? Something that is released as soon as the kidney is damaged, that is sensitive and specific, that can return a result quickly, that has levels that change in proportion to the degree of damage or the likelihood of recovery, and preferably, that can be detected noninvasively, ideally in the urine. This would remove the need for costly and unpleasant kidney biopsies, and reduce the reliance on blood samples.

THE DIFFERENT TYPES OF KIDNEY DISEASE AND DAMAGE

The role of the kidneys is to filter fluid and waste out of the blood, and kidney disease or damage is most simply defined as the loss of kidney function, correlating to the rate that fluid is filtered out of the kidney, called the glomerular filtration rate (GFR).

Acute kidney injury

Acute kidney injury (previously known as acute renal failure) is a sudden loss in kidney function. There are a number of triggers, including blood and fluid loss from accident, dehydration, burns, or surgery, side effects from drugs, cardiovascular disease, infection, liver failure or severe allergic reaction[1]. Avoiding drug-induced acute kidney injury is an important driver in drug development for a wide range of diseases.

Early-stage kidney disease often has no clear symptoms, and so physicians may miss it until it progresses into its later stages.

"Acute kidney injury happens very quickly; … the kidney can shut completely down in just one to two days," says Timothy Carlson, senior director of Laboratory Services and Biomarker Innovation at Pacific Biomarkers, a biomarker laboratory services provider that supports preclinical and Phase I-IV studies in drug development.

Chronic kidney disease

Chronic kidney disease (also known as chronic renal disease) is a more gradual loss of kidney function,[1] most often triggered by diabetes and hypertension. Cardiovascular disease, obesity, and increased cholesterol levels also play a part, as do repeated episodes of acute kidney injury.[2]

In the U.S. in 2005-2010, 14% of the adult NHANES (National Health and Nutrition Examination Survey) population had chronic kidney disease, increasing from 12% in 1988-1994. The incidence of chronic kidney disease has risen from 25% to 41% in people with cardiovascular disease.[3]

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THE LIMITATIONS OF EXISTING BIOMARKERS

The main biomarkers used to estimate GFR are serum creatinine and BUN. Creatinine is a metabolite from the breakdown of muscle. The kidneys filter creatinine out of the blood, and so serum levels can reflect how well the kidneys are working. Doctors have used serum creatinine as a biomarker for renal function for almost 100 years, and while it is useful, it does have its shortcomings. Creatinine production can be affected by factors unrelated to kidney disease, such as race, age, sex, menstrual cycle, diet (including how much protein someone eats and how it is prepared), and even how muscular someone is. Medications can also affect the rate of creatinine secretion.[4,5]

Another issue with serum creatinine is the timescale: Once GFR begins to fall, it can take several days for this to show up in the serum creatinine levels, by which time the damage has started to set in. This makes it hard to use as a marker of progression.

BUN is another well-established biomarker. The liver converts waste products, including nitrogen from dietary protein breakdown and tissue turnover, into urea. This circulates in the blood to the kidneys, where it is excreted. The levels of urea in the blood can therefore act as an indicator of how well the kidneys are working. A number of factors can affect these levels, including dehydration, dietary protein intake, heart disease, medications, fever, trauma, pregnancy, and liver disease.[5]

Diabetic kidney disease is spotted by measuring the ratio between albumin and creatinine levels in the urine, but at early

stages, the levels are very low, making them difficult to detect.

The limitations of these established markers highlight the need for new biomarkers specifically for kidney disease, and interest in biomarkers of renal injury has indeed increased over the past 10 years, according to Carlson.

EMERGING BIOMARKERS: WORKING TO MEET THE NEED

Some of the many renal biomarkers that have been assessed over recent years are listed in Table 1. However, none of these has performed much better than the traditional measures in clinical studies, as Paul Kimmel, senior adviser in the Division of Kidney, Urologic and Hematologic Diseases at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), part of the NIH, explains: "It's important to look at putative biomarkers in a clinical context and consider whether the marker will meet the clinical need—whether it is 'fit for purpose.' "

BIOMARKERS DRIVING DRUG DEVELOPMENT FORWARD

Much of the focus on renal biomarkers has been in drug development rather than in diagnosis. Drug development is a high-risk business, and despite rising levels of investment, drugs are still failing late on in development. Robust and validated biomarkers are vital for reducing potential kidney damage caused by drugs developed for nonrenal indications, as well as the development of drugs for acute kidney injury and chronic kidney disease.

"Biomarkers should be developed as early as possible in the drug development process, for example to stratify patients in clinical trials and reduce the drug failure rate," says Peter Tunon, vice president of personalized medicine at 20/20 GeneSystems, a company with a focus on biomarker-based companion diagnostic assays. "Companion diagnostics based on biomarkers are also likely to become significant for drug approval and in reimbursement."

Biomarkers helping reduce drug-induced kidney damage

One of the key reasons behind late-stage and costly drug development failures is kidney injury, but existing markers aren't sensitive enough to detect this earlier. Being able to spot the potential for kidney injury early on in drug development could trigger money- and time-saving go/no go decisions for pharmaceutical companies, which would provide knock-on benefits for patients by resulting in safer and better drugs, potentially at a lower cost.

The European Medicines Agency (EMEA) and the U.S. Food and Drug Administration (FDA) jointly qualified a panel of 7 biomarkers to act as surrogate markers for drug-

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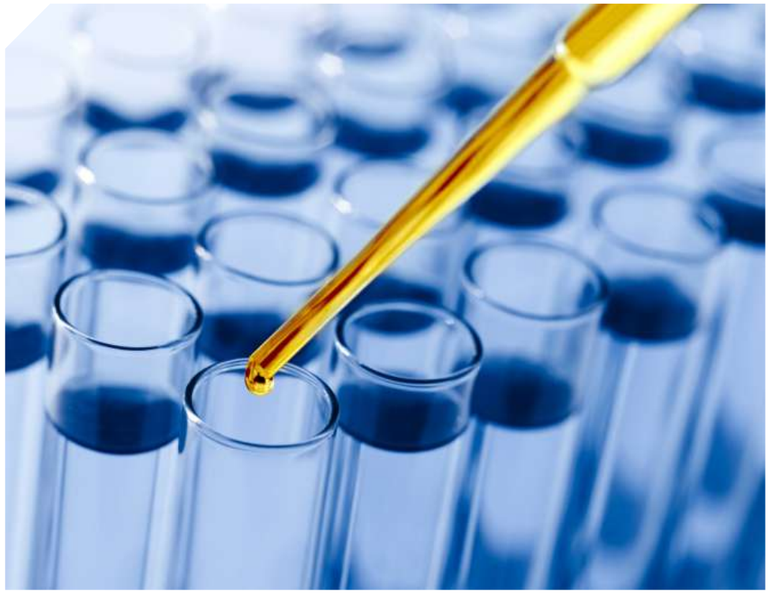

The "Fit-for-Purpose" Validation Approach in Renal Injury Biomarker Assays

BY ADAM AULBACH, DVM, DACVP, DIRECTOR OF CLINICAL PATHOLOGY AND PRINCIPAL CLINICAL PATHOLOGIST

▶Drug-induced kidney injury (DIKI) remains one of the most common causes of new drug attrition today. In 2008, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) announced that they would consider the results of seven novel renal injury biomarkers (KIM-1, albumin, total protein, β2-microglobulin, Cystatin C, Clusterin, and Trefoil Factor-3) in the context of preclinical drug development and renal safety evaluation. Since then, the characterization and identification of renal injury biomarkers continue to expand, largely due to the efforts of organizations such as the Predictive Safety Testing Consortium (PSTC), the Health and Environmental Sciences Institute (HESI), and many of the major pharmaceutical companies. It has become clear from these efforts that the sensitivity and specificity of many newer renal biomarkers are far superior to traditional serum and older-generation urinary analytes (e.g., BUN, creatinine, NAG, GGT) in identifying early and subtle DIKI; however, it remains important to recognize the limitations of these assays and their appropriate application in preclinical safety studies.

The value of these novel renal injury biomarkers has been consistently demonstrated through the use of biological validations using a variety of nephrotoxicity models in animals.

- Many of these biomarkers show positive signals before perturbations in serum-based renal injury markers, positive signals which often correlate with the first onset of microscopic tissue changes, traditionally considered the gold standard for identifying renal injury.

- This allows for more rapid identification of drug liabilities, accelerates decision making in the drug development process, and minimizes lost resources due to drug attrition.

- A more comprehensive evaluation of renal injury may be accomplished through individual biomarkers that have been developed to localize injury to specific areas of the kidney, including the proximal and distal tubules, collecting duct, glomerulus, and the renal papilla.

Evaluating a panel of biomarkers at appropriate time intervals is recommended to accurately characterize the renal injury profile specific to the compound of interest. This targeted approach is best designed by scientific personnel who are not only familiar with the assays themselves, but also possess a working understanding of renal pathology and physiology.

Most of the assays for these novel biomarkers are antibody-based immunochemistry assays that have not yet been standardized across the preclinical industry. Numerous methods and commercially available kits have been developed for many of these analytes; however, method-to-method variation and the lack of continuity from species to species continue to be obstacles. Although antibody targets for these assays have been clearly identified, the assays themselves may vary considerably in their sensitivity, specificity, dynamic range, baseline values, and overall performance. While there are currently no rigid requirements for the validation of such assays, many scientists involved in this work apply some form of the "fit-for-purpose" validation scheme, which is increasingly finding its way into industry guidance documents. Essentially, the "fit-for-purpose" approach states that each individual assay should be validated to a sufficient degree equivalent to the level of decision-making power that will be applied to the results. ●

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induced renal toxicity. This was based on data from the Predictive Safety Testing Consortium (PSTC), a public-private partnership between industry, academia, and regulators, and founded by the nonprofit Critical Path Institute. According to the EMEA, this was the first time that the biopharma industry had pooled data from different companies for such a project.[11]

"Drug development has been enabled by the qualification of this renal toxicity panel," says Pike. "Approaches like this will allow physicians to tailor drug regimens to patients, finding the optimum therapeutic window that balances efficacy and safety to provide the best outcome."

New biomarkers need to be screened and qualified to assess their potential in spotting drug-induced acute kidney injury.

Table 1: Biomarkers linked with kidney injury[6, 7, 8]

Alkaline phosphatase	Alpha-1 microglobulin	Alpha-2-macroglobulin
Angiopoietin-related protein 3	Angiotensin-converting enzyme	Beta-2-microglobulin
Calbindin	Carbamylated hemoglobin	Clusterin
Complement C3	Complement factor H-related protein 1	Connective tissue growth factor
Creatine kinase-MB	Creatinine	Cystatin C
Epidermal growth factor	Epidermal growth factor receptor	Erythropoietin
Gamma-glutamyl transpeptidase	Gamma-glutamyltransferase	Glutathione S-transferase alpha
Glutathione S-transferase Mu 1	Interleukin 6 (IL-6)	IL-8
IL-10	IL-18	Interferon-gamma-induced protein 10
Kidney injury molecule-1 (KIM-1)	Lactate dehydrogenase (LDH)	Matrix metalloproteinase-9 (MMP-9)
Microalbumin	Monocyte chemotactic protein 1	Myoglobin
N-acetyl-β-D-glucosaminidase (NAG)	Neutrophil gelatinase-associated lipocalin (NGAL)	Neutrophil CD11b
Osteopontin	Prohormone of atrial natriuretic peptide (ProANP)	Retinol-binding protein
Sodium/hydrogen exchanger 3 (NHE3)	Tamm-Horsfall urinary glycoprotein	Trefoil factor 3
Vascular endothelial growth factor		

However, no one group can do this alone. As Carlson explains, Pacific Biomarkers is working with regulatory bodies, academia and a range of biopharma companies that are part of the PSTC to qualify a panel of a half-dozen biomarkers that could detect kidney damage early, or even predict it before it happens.

The PSTC, in collaboration with the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium, is running a two-year clinical study to advance the acceptance of new biomarkers designed to detect drug-induced kidney injury in clinical trials. This launched in November 2011. The study is looking into biomarkers that are able to identify early-stage drug-induced kidney injury accurately and sensitively. The aim is to develop new biomarkers with potential to monitor drug safety in clinical trials, as well as improve the diagnosis of kidney injury in patients treated with a range of different drugs.[9]

"Patient safety is and must be our primary concern as we develop potential new medicines," explained Gary Herman, vice president of Early Stage Development at Merck Research Laboratories. "The FNIH Biomarkers Consortium Kidney Safety project is critical to help identify biomarkers that improve the process of developing effective medicines that are safe to test and use with patients."

The project's initial focus was on cisplatin, a cancer chemotherapy known to affect the kidney tubule, and aminoglycosides, antibiotics used in cystic fibrosis.

Rules-Based Medicine (now part of Myriad RBM) has launched a 16-biomarker Human Kidney Multi-Analyte Profile (Human

Kidney MAP) biomarker testing service, developed in response to the FDA's Critical Path initiative and designed to help biopharma companies understand more about drug toxicity by detecting damage early and pinpointing its location within the kidney.[10]

As Dominic Eisinger, director of strategic development at Myriad RBM, explains, Human Kidney MAP was the next logical step of translating the utility of the FDA- and EMA-qualified biomarkers of rat kidney damage to human clinical utility.

"All of the markers [in Human Kidney MAP] were identified by the PSTC as potentially improving upon the existing measures of kidney toxicity, serum creatinine, and BUN. No single marker can be applied universally across many possible renal injury contexts. Measurement of all the renal injury markers measured in parallel enables one to capture critical information with regard to renal toxicity, repair, and function, from study start to finish," says Eisinger. "Human Kidney MAP is routinely integrated in drug development strategy, serving as an indicator regarding range and interval of dosing. In addition, preclinical rat kidney data may permit the development of a drug previously not viable, since the Human Kidney MAP can detect and monitor early renal injury. We believe that using these biomarkers can make drugs safer and help in the identification of candidates for a given treatment."

Renal biomarkers underlying kidney disease treatments

There has been less of a focus on chronic kidney disease compared with acute kidney injury, and biomarkers are much less well developed in this area.

"Chronic kidney biomarkers are very much in their infancy," says Kimmel. "This is partly because

the onset of chronic kidney disease is slow and insidious, so it is hard to delineate where it begins, and partly because it has been hard to find markers that beat the existing diagnostic, the measure of levels of protein in the urine."

The NIDDK supports the CKD Biomarkers Consortium, which is working to promote the discovery and validation of biomarkers in chronic kidney disease. A number of companies are developing biomarkers for use in drug development for different forms of kidney disease, and these are supported by the advance of new technologies, such as combining mass spectrometry with metabolomics, as in ClinMet's approach.

Focusing on diabetic kidney disease, ClinMet combines urine metabolome data with clinical, biochemical and computational analysis to create a metabolomic signature, based on research carried out at the Clinical and Translational Research Institute and the Institute of Metabolomic Medicine at the University of California, San Diego.

"Metabolomics provides a higher yield of important biomarkers," says Sharma. "We have focused on urine—urine testing is noninvasive and easy for both patients and healthcare professionals—but blood profiling can be added to give a fuller picture."

ClinMet's mass spectrometry-based theranostics platform can be used to screen new drugs. This includes assessing efficacy, looking for off-target effects, calculating the correct dose, predicting toxicity, or monitoring patients' improvement in clinical trials. It also serves as a diagnostic and monitoring tool, and helps to understand more about a drug's mechanism of action.

"We are using renal biomarkers to help us understand the science related both to the drug and to the disease. We believe that this is a critical step needed to advance the field," says Sharma. "We have been able to link the markers that we have found using mass spectrometry with changes in urine and in biochemical pathways, and we have changed the way we think about diabetic complications. As well as tracking disease, biomarkers can provide insight."

There is crossover between drug-induced kidney damage and kidney disease research, however. As McDade explains, the Myriad RBM panel is a kidney-damage panel and not a toxicity panel, and so has a role in areas such as diabetic kidney disease and lupus: "We are initiating studies in these areas and working on validating our panel for these indications."

Myriad RBM is collaborating with Sanofi ($SNY) to find protein biomarkers as part of Sanofi's Outcome Reduction with Initial Glargine Intervention (ORIGIN) study, a trial in pre- and early diabetes, and with DaVita Labs to carry out protein biomarker discovery research to develop diagnostic tests to support the treatment of dialysis patients.

PREDICTIVE AND DIAGNOSTIC RENAL BIOMARKERS

Renal biomarkers play a role in diagnostics beyond the clinical trial, for example in diagnosis, treatment monitoring and in personalized medicine.

"Biomarkers are diagnostic, prognostic or predictive. Diagnostic biomarkers could allow early identification of people at most risk for sustaining injury, allowing drug administration at the right stage of their disease, when it would be the most beneficial. Prognostic biomarkers would allow the identification of people with kidney damage that is treatable

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IAN PIKE, COO, PROTEOME SCIENCES

or irreversible, and predictive biomarkers could be used in personalized medicine, to pick out those patients most likely to respond to specific drugs," says Kimmel.

Picking out the patients at risk

Knowing which patients are most at risk of developing certain forms of kidney disease means that physicians can keep a close eye on them, allowing treatment at a much earlier stage. Because of the complexity of the disorder, this would probably require a panel rather than a single marker.

Proteomics International, an Australian proteomics analytics and service company, has created a biomarker discovery platform and in a pilot study has been able to identify and validate a panel of 13 potential protein biomarkers related to diabetic nephropathy. This could be the basis of a specific and selective diagnostic, as Richard Lipscombe, founder and managing director, explains: "The challenges in searching for biomarkers are to obtain detailed individual data to remove potentially confounding variables when looking at associations. Analysis of clinical samples has identified protein biomarkers for diabetic kidney disease including proteins involved in metabolism, inflammation and oxidative stress."

The international GENIE (GEnetics of Nephropathy: an International Effort) consortium has found that two single-nucleotide polymorphisms (SNPs--single letter changes in the DNA code) could affect patients' risk of developing diabetic kidney disease (diabetic nephropathy).

Renal biomarkers in treatment monitoring

Once drugs have made it to the market, renal biomarkers are also used to monitor treatment.

"For some powerful drugs, such as cancer agents and antibiotics, there is a balance between risk and benefit, with doctors and their patients prepared to take the risk of adverse events such as reversible acute kidney injury. Renal biomarkers could be used to track the severity of kidney injury and signal the need to discontinue administration of drugs such as cisplatin," says Carlson.

Screening patients for kidney disease as a routine part of their care, whatever treatment they are receiving, could improve their overall health. NICE (the National Institute for Health and Care Excellence) in the U.K. has recommended that all hospital patients be checked for acute kidney injury. This affects up to one in 6 patients admitted. Ensuring that acute kidney injury is prevented, even by simple acts such as ensuring good hydration, has potential to save the U.K.'s NHS (National Health Service) millions of pounds a year, as well as saving up to 12,000 lives.[12]

Personalized medicine

Biomarkers have the potential to bring personalized medicine to renal disease, as McDade explains: "We believe biomarkers will play a critical role in identifying patients for the correct therapy based on their genetic predisposition, monitoring therapy response, predicting long-term outcomes, and diagnosing disease."

Renal damage biomarkers could also potentially be used to take a personalized approach to other diseases.

"Using new biomarkers to monitor renal function in patients receiving both broadly cytotoxic agents as well as more targeted medicines will be critical in optimizing individual outcomes. Each patient will manage drug and metabolite excretion differently with corresponding variation in renal safety. This will be particularly important in chronic dosing for conditions such as arthritis and dementia," says Pike.

THE FUTURE

A lot of work is still needed in the renal biomarkers field, but ongoing research, and improving focus in this area, particularly from the consortia that are bringing together experts from both industry and academia, will move things forward both in acute and chronic kidney disease. An increasing use of technology such as mass spectrometry, along with all the omics, will speed discovery and validation.

Kimmel has great hopes for the next half-decade of renal biomarker research: "Over the next 5 years, I expect to see a better delineation of diagnostic biomarkers and a move in the field from diagnostic to prognostic biomarkers, especially in the treatment of kidney disease. The genetics of kidney disease should become clearer, which will help us to identify susceptible patients at a much earlier stage. And finally, I expect to see more clinical trials using kidney biomarkers, including combinations of biomarkers, making drug development faster and safer." ●

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