A guidance for renal biomarker lead optimization and use in translational pharmacodynamics

Josef S. Ozer

Pfizer Global Research and Development, Pharmacokinetics, Dynamics, and Metabolism, St Louis, MO 63017, USA

Guidance for the use of biomarkers in pharmaceutical development and clinical trial optimization will reduce developmental cycle time. A 'fit-for-purpose' guidance for biomarker use is considered herein when the same biomarker is applied in very different contexts in drug development and after regulatory approval. Recent approved use of renal safety biomarkers in Good Laboratory Practice studies lacks sufficient guidance for the use of these markers across the drug development pipeline. In lead optimization, renal injury biomarkers are possible anchors for promising new prodromal metabolic biomarkers, which are applied before lead candidate selection. Renal injury biomarkers can now be evaluated as potential efficacy and pharmacodynamic biomarkers in clinical trial proof-of-concept studies for diabetic nephropathy.

Regulatory qualification of renal safety biomarkers

The recent qualification and regulatory acceptance of a set of urinary renal injury markers for preclinical testing and decision-making in Good Laboratory Practice (GLP) studies is a historic milestone for the pharmaceutical, medical, academic and regulatory communities [1,2]. The first voluntary eXploratory data submission (VXDS) was sponsored by the Predictive Safety Testing Consortium (PSTC). The use of kidney injury molecule-1 (Kim-1), trefoil factor 3 (TFF3), clusterin and albumin to monitor drug-induced acute kidney tubular injury and β2-microglobulin, cystatin C and total protein to monitor drug-induced acute glomerular injury with proximal tubular involvement in rat GLP studies marks a novel partnership between broad spectrums of the medical community to improve the tools for drug development and in a concurrent effort, an additional VXDS application for the qualification of glutathione-*S*-transferase (GSTα) and additional biomarkers was submitted by The Health and Environmental Sciences Institute of the International Life Sciences Institute (ILSI/HESI) (Table 1) (personal communication, Sven Beushausen). Our focus is on the next application of these markers to other crucial steps in the translational drug development process, including lead optimization approaches, therapeutic efficacy and pharmacodynamics, and associated modeling with pharmacokinetic (PK) data.

Lead optimization of renal hemodynamic changes early in drug development in the pipeline

Novel approaches are recommended herein to reduce the incidence of renal injury observed early in drug development preceding traditional dose-escalating safety studies in rodents. Reduced cycle time in drug development is a continuous goal of the pharmaceutical discovery process, but resource limitations may influence the implementation of these strategies. A principal goal for the pharmaceutical processes is to identify and use a crucial marker at the earliest period in development to render a decision to proceed to the next stage gate of the development process. The use of lead optimization strategies to detect potential or prodromal renal events earlier in the drug discovery pipeline enables rapid decision-making and provides opportunities for chemistry support to redesign drug structure to avoid potential renal events. Current safety assessment detection of treatment-induced renal injury in studies with limited margins of efficacy could suggest that a program be suspended unless a backup compound demonstrates normal renal function at the efficacious dose range. Knowledge gained by screening earlier against renal injury enables selection criterion beyond traditional efficacy and target mechanism selection and safety assessment criterion. Lead

☆ The personal views expressed in this article by the author may not be understood nor quoted as being made on behalf of or reflecting the position of the FDA, EMEA, or ILSI/HESI, PSTC, or IMI and its membership.

E-mail address: josef.ozer@pfizer.com.

1359-6446/06/$ - see front matter © 2010 Published by Elsevier Ltd. doi:10.1016/j.drudis.2009.12.001

TABLE 1

VXDS renal biomarkers (PSTC and ILSI/HESI sponsored)

Renal biomarker	Qualified (rat GLP)	Exploratory (FIH)	Injury anatomy
Kidney injury molecule-1	Yes	Case by case	Tubular
Clusterin	Yes	Pending	Tubular
Albumin	Yes	Case by case	Tubular
TFF3	Yes	Pending	Tubular
β2-Microglobulin	Yes	Case by case	Glomerular
U-cystatin C	Yes	Case by case	Glomerular
Total protein	Yes	Case by case	Glomerular
GSTα	Pending	Pending	Tubular
S-cystatin C	Recommend	Propose	All, functional
NAG	Recommend	Propose	Tubular
NGAL	Recommend	Propose	Tubular
OPN	Recommend	Propose	Tubular

Abbreviations: GSTα, alpha-glutathione-*S*-transferase; NAG, *N*-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; OPN, osteopontin; S, serum; U, urine. Pending data generation indicates that a data package is being assembled for potential VXDS submission at a later date, [1,2] and note added in proof.

optimization approaches require higher throughput and rapid turnaround, making time-intensive histopathology approaches ill-suited to the time demands in standard drug development approaches, although exceptions could be considered. Historical and well-developed tools in absorption, distribution, metabolism and excretion (ADME) and pharmacokinetics departments in the pharmaceutical discovery process could be adapted to address this problem in a lead optimization model in advance of traditional safety assessment approaches. Drug development programs with known or anticipated renal liability at target identification would greatly benefit from this proposed path forward. At this time, it is too preliminary and costly to implement similar strategies across other programs in the pharmaceutical pipeline without a known potential renal liability at target selection. Nevertheless, successful implementation of lead optimization strategies might eventually impact a broader sector of programs over time, if proven successful.

Cyclosporine treatment induces renal hemodynamic changes in the absence of histopathologic alterations

Some therapeutic treatments provide great medical benefit even with known toxicological risks to patients, which are closely monitored and managed by the clinician. One notable example is cyclosporine (CSA) treatment to prevent rejection in many types of organ transplantation. Even a single dose of CSA can produce renal hemodynamic changes in patients who are accompanied by significant reductions in glomerular filtration rate (GFR), a commonly accepted clinical measure of renal functional decline [3]. Patients on CSA therapy reveal a narrow therapeutic margin, and observed renal toxicity is dependent upon formulation [4]. PK studies enable CSA exposure levels to be appropriately assessed clinically and monitored compared with random trough determinations [5]. Studies suggest that CSA nephrotoxicity in patients is irreversible and is progressive, even when dosing is closely monitored, leading to renal interstitial fibrosis after 6–12 months [6,7]. Thus, the development of newer immunosuppressive therapies is a great therapeutic need for transplantation patients, the medical community and pharmaceutical discovery. Chronic CSA treatment in rat models has demonstrated adverse hemodynamic effects in the absence of associated structural abnormalities (i.e. histopathology) [8]. Thus, evidence suggests that labor-intensive histopathologic approaches might have broader limitations as a benchmark assay for certain developmental programs, especially lead optimization approaches. Novel therapies supporting organ transplantation and immunological disorders are being developed by leading pharmaceutical and biotechnology companies and require close evaluation for renal injury early in discovery and preclinical development to avoid potential safety issues late in clinical development, when the costs of termination are exceedingly high.

A 24-hour study model adapted to monitor hemodynamic renal change from exposure to new chemical entities

Lead optimization assessment in the adaptation of the commonly employed PK study paradigm provides multiple blood samplings in a short period, 24–72 hours. Plasma volumes are limited, although compound determination for a PK curve uses just a portion of the sample isolated. A limited biomarker measurement might be assessed in an analytical platform such as liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) with the limited remainder of samples collected. At study termination, additional plasma volume can be collected for traditional clinical chemistry and antibody-based biomarker endpoints. Urine samples can also be collected for the full 24-hour study duration for further biomarker analysis. Current renal toxicity assessment focuses on urinary biomarkers, and those candidate biomarkers could be evaluated using urines collected in this study paradigm. Dosing to explicit renal injury endpoints might require a modified Institutional Animal Care and Use Committee (IACUC) protocol compared with the traditional PK study.

A potential concern for a successful lead optimization approach is a multiplicity of biomarker platforms used to evaluate biomarkers. MesoScale Discovery, or MSD (ELISA-like), and Rules-Based Medicine, or RBM (Luminex beads), detect urinary acute kidney injury biomarkers in a multiplexed approach (Tables 1 and 2), yet the time to data acquisition from these antibody-based platforms

would be somewhat longer compared with generating both PK and biomarker data from LC/MS/MS in parallel. Thus, the use of multiple platforms for biomarker analysis might delay a lead optimization process.

Whether urinary renal injury biomarkers could detect renal injury or functional changes in a 24-hour study paradigm might be governed by escalating treatment dose. Although the 24-hour time point is not typically tested in current safety assessment practices, it is recommended that future studies apply qualified renal biomarkers to this study paradigm. Whether an early renal biomarker signal is to be considered prodromal (symptomatic) of injury or false positive in the absence of concordant histopathology data requires further studies and alignment among pathologists and physiologists. We recommend that the 24-hour study paradigm be considered for such investigations because it is an optimal application in the early pharmaceutical discovery and development process. Discovery of renal toxicity in GLP models precludes rapid project rebound and recalibration from such observations.

Cysteinyl leukotrienes and hepatobiliary dysfunction as lead optimization biomarker tools to monitor renal injury from novel immunosuppressant entities

CSA is a profound immunosuppressant for use in organ transplantation and immune-mediated illnesses, but the potential risk of renal injury makes the therapeutic monitoring of patients complex and costly. Patient variability in PKs is dependent upon organ transplant type, disease state and age, making optimized trough determinations difficult to calculate [9]. Pharmaceutical concerns are evaluating novel targets to discover improved small- and large-molecule therapies currently treated by CSA. Novel compound chemistry and leads for these indications are candidates for renal injury assessment in a lead optimization strategy. Systemic CSA dosing was administered in a short-duration study paradigm, in which renal-derived cysteinyl leukotriene (LTC_4 and LTD_4) elimination was studied with GFR and bile flow excretion [10]. Hepatobiliary dysfunction at nephrotoxic CSA treatment was revealed by impaired LTC_4 and LTD_4 elimination through the bile duct, which leads to increased renal tissue levels. Systemic administration of LTC4 and LTD4 also induces GFR and real function parameter reductions [11]. CSA-induced toxicity can be partially averted by coadministration of an LTC_4 and LTD_4 receptor antagonist, indicating a direct role of LTC_4 and LTD_4 renal accumulation as a basis of observed nephrotoxicity [12]. Complete block of CSA-induced renal toxicity is observed by coadministration of a LTC_4 and LTD_4 receptor antagonist and a TxA_2 receptor antagonist [12]. CSA-induced increases of LTE_4 and *N*-acetyl-LTE_4, which are metabolic endpoints for LTC_4 and LTD_4 levels, have been demonstrated in urine [13]. As a mediator of CSA-induced vasoconstriction, TxA_2 elevation can be monitored by detecting its rapid conversion to urinary TxB_2 (renal synthesis) and TxB_2 metabolite 2,3 dinor TxB_2, which indicates systemic synthesis [14].

Assessment of individual bile acid elevations in a lead optimization model

Because bile flow excretion is impaired with CSA administration [10], it has been postulated that individual bile acids are elevated in urine and plasma (Table 2). IV administration is recommended in lead optimization to avoid possible absorption issues with oral dosing within the first four hours of dosing. Fasting is required to deplete steady-state levels of bile acids in blood from regular dietary intake [15]. Because IACUC regulations require resumption

TABLE 2

Renal lead optimization biomarkers proposed

Renal biomarker	Rat qualified (GLP)	Prodromal	Assay platform
U-kidney injury molecule 1	Yes	Yes	Luminex, MSD
U-LCN2	Yes	TBD	Luminex, MSD
U-clusterin	Yes	TBD	Luminex, MSD
U-β2-Microglobulin	Yes	TBD	Luminex
U,S,P-cystatin C	Yes, Recommend	Functional Δ	Luminex
U-GSTα	Pending	TBD	Luminex
NAG	Recommend	Yes	Clinical analyzer
U-cathepsin B	No	TBD	Clinical analyzer
U-creatinine	Historic benchmark	TBD	Clinical analyzer
U-trefoil factor 3	Yes	TBD	LC/MS/MS, EIA
S,P-bile acids	No	TBD	LC/MS/MS
U-bile acids	No	TBD	LC/MS/MS
U-LTE4	No	TBD	LC/MS/MS
Renal tissue LT panel	No	TBD	LC/MS/MS
S,P-HETE panel	No	TBD	LC/MS/MS
S,P-creatinine	Historic benchmark	TBD	LC/MS/MS
S,P-citrulline	No	TBD	LC/MS/MS
U-TxB_2 and panel	No	TBD	LC/MS/MS

Abbreviations: U, urinary; S,P, serum or plasma; LT, Leukotrienes; Δ, change; TBD, to be determined. Prodromal indicates a biomarker signal that is symptomatic of injury that precedes a histopathology or an accepted clinical chemistry anchor [35]. Ra qualified for GLP [1,2] and note added in proof.

of normal feeding at four-hour post-dose, bile acid elevation from CSA hemodynamic change should be assessed within this time window (zero to four hours). CSA-induced perturbations of leukotriene elimination through the bile duct were observed 90 min post-dose [10]. Fasting time could be bracketed differently before therapeutic dosing. Exploratory work will assess whether an individual bile acid, a subset of bile acids or total bile acids are sufficient for monitoring hemodynamic changes with CSA treatment.

Integration and analysis of biomarker endpoints for autoimmune therapeutics in a lead optimization paradigm

At zero to four hours, plasma bile acid elevations are considered a crucial biomarker signal in this paradigm (Table 2). With leukotriene accumulation in the kidney, urinary LTE_4 and *N*-acetyl-LTE_4 elevations are anticipated because these metabolites are at the terminus of the leukotriene biosynthetic pathway and are another key biomarker for this approach [13,16] (Table 2). LTE4 and *N*-acetyl-LTE_4 elevations depend upon whether alternative renal clearance pathways are available and functional. At study completion, leukotriene elevations from treated renal tissue extracts are anticipated (LTC_4 and LTD_4). From 0 to 24 hours, plasma 5-HETE elevation is considered an exploratory biomarker to examine (Table 2). Elevations of urinary TxB_2 metabolites are expected to corroborate with renal tissue LTC_4 and LTD_4 elevations but are considered more exploratory [14] (Table 2). Serum creatinine can be monitored by LC/MS/MS because sample volume is limiting during the collection period and insufficient for clinical chemistry, except for the necropsy sample (Table 2). Serum creatinine elevation is not anticipated, although a functional elevation cannot be ruled out as a possibility in this short 24-hour study model. Serum citrulline can be measured with creatinine in LC/MS/MS, and reports indicate that it elevates two-fold in patients with mild renal insufficiency (GFR < 15 mL min^{-1} 1.73 m^{-2}) [17]. Urinary creatinine could be measured by LC/MS/MS or clinical chemistry to normalize urinary renal biomarker marker determinations. Novel renal markers of acute kidney injury would be assessed by MSD or RBM platforms (Kim-1, cystatin C, TFF3, β2-microglobin, clusterin, osteopontin, GSTα and albumin) (Table 1), although multiplexing by novel MS approaches would be highly price-competitive in high-throughput mode [18]. An advantage of MS is technical translation that is facilitated across species, although antibody approaches might be limited, especially for biomarkers in mouse (Table 2). A high percentage of discovery programs use mice, making MS approaches attractive to use, especially with small volume (nano) applications. We envision that one platform, LC/MS/MS, would ultimately reduce the cycle time of the lead optimization paradigm.

Specificity for lead optimization of organ functional change and injury

We propose that successful lead optimization approaches for liver and renal injury might reduce cycle time and the number of compounds detected for organ injury in GLP safety assessment studies. A novel approach to monitor CSA-induced renal hemodynamic toxicity in a lead optimization paradigm has been outlined. Profound reductions in drug development cycle time might be achieved by the companies using similar approaches. Novel and traditional acute kidney injury biomarkers [1,2] are candidate anchors for CSA-induced leukotrienes and bile acids measured on a high-throughput LC/MS/MS platform (Tables 1 and 2). This proposed platform could also be applied for liver injury lead optimization with modifications. Additional measurement of bilirubin and metabolic modulators of liver function makes this an equally powerful tool to help manage a toxicity that is a leading cause of developmental attrition and responsible for a high incidence of post-market withdrawals [19,20]. The development and implementation of novel lead optimization approaches for organ injury enables pharmaceutical companies to devote greater resources to novel target selection and development of improved efficacy markers that are predictive and reliable surrogates of well-defined clinical endpoints. The focus on renal lead optimization strategies here does not imply that this screening approach is the most vexing issue for immunotherapy programs to address. Other target, mechanistic and selectivity issues are also of crucial importance for pharmaceutical project teams.

Renal biomarkers of compound efficacy and pharmacodynamic modeling

Urinary albumin is the gold standard biomarker for therapeutic efficacy in diabetes-induced renal nephropathy. Emerging data show that reduction of excreted albumin (albuminuria) leads to reduced adverse renal and cardiovascular risk [21,22], although the reduced cardiovascular risk will not be addressed further in this review. Dipstick measurements of albumin are not sufficiently sensitive for efficacy biomarker endpoints [23]. Microalbuminuria is defined as 30–300 mg excreted per day, according to the American Diabetes Association [24]. Major trials linking microalbuminuria to renal outcome in diabetes patients include the Irbesartan Diabetic Nephropathy Trial (IDNT), the Reduction of Endpoints in Non-Insulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan study (RENAAL), and the Microalbuminuria Reduction with Valsartan trial and Irbestartan in Patients with Type 2 Diabetes and Microalbuminuria [21]. In the IDNT trial, therapeutics lowered the risk of creatinine doubling (~35%) and end-stage renal disease (23%) and correlated with reduced proteinuria [25]. In RENAAL, halving albuminuria decreased end-stage renal disease by half with losartan therapy [26]. No correlations have yet been made in population studies between albuminuria and GFR during the onset of diabetic nephropathy, indicating that additional biomarkers of renal injury should be considered for therapeutic efficacy determinations [27].

Albumin excretion rate identifies most, but is not prognostic for all, type 2 diabetics

For overt type 2 diabetes, distinct albuminuric (75%) and nonalbuminuric (25%) pathways reveal a decline in GFR (<60 mL min^{-1} 1.73 m^{-2}) [27,28]. Although the majority of patients progress from normo to microalbuminuria with a static GFR, a sizable number of patients show a declining GFR and normoalbuminuria levels, a relationship that is independent of renin angiotensin inhibitors and intrarenal vascular disease [27,29]. A smaller subgroup of patients showed microalbuminuria while maintaining GFR (>60 mL min^{-1} 1.73 m^{-2}) [27]. Meta-analysis of 33 antihypertensive studies indicates that the albumin–GFR

relationship exists for late nephropathy but not early nephropathy [27]. Thus, chronic kidney disease (CKD) stages 1 and 2 require a balance of GFR and albumin excretion data for decision-making purposes. The panel of acute kidney injury biomarkers should be examined in this same model (Table 1). Biomarkers that add information to the nonalbuminuric patients would be of the greatest value. Other renal biomarkers have been characterized recently for diabetic renal injury and might also add value to albumin determinations in an efficacy model of a therapeutic candidate.

Urinary cathepsin B adds value to albuminuria in assessing type 2 diabetes

The renal proximal tubule contains large stores of lysosomes that contain specific enzymes including urinary cathepsin B (CB) and *N*-acetyl-β-D-glucosaminidase (NAG). Damage to the proximal tubule causes the release of these enzymes, which is specific to kidney [30]. The enzymatic activities are measured using analytical measurements in clinical chemistry platforms. Type 2 diabetics ($n = 130$) and controls ($n = 42$) were evaluated for urinary albumin, CB and NAG elevations, which were correlated to glycemic control [31]. Diabetics showed a range of albumin levels, mostly microalbuminuria; however, approximately 10% of the patients showed normoalbuminuria [31]. Normalized urinary CB elevations were approximately three-fold elevated compared with controls in these patients, and levels elevated to six-fold in patients with microalbuminuria [31]. CB elevations correlated with patient plasma glucose concentration, which increased ($r = 0.62$) in patients with poor glucose control (>9.7 mmol/L) [31]. NAG elevations correlated more closely with albuminuria but not glycemic control. CB is a novel biomarker of renal nephropathy that adds value to albuminuria, which is considered the gold standard for diabetic nephropathy [23]. In type I diabetics (mean age 15 years), high urinary NAG and retinol-binding protein elevations were observed in microalbuminuria patients and approximately two-fold elevations in normoalbuminuria patients [32]. Additional clinical evidence is required to further substantiate these interesting observations.

Serum cystatin C adds value over creatinine-estimated GFR in identifying mild type 2 diabetes

Because type 2 diabetic patients in the normo- to microalbuminuria range might not show a static GFR, a more accurate assessment of estimated GFR would be an additional tool for clinical endpoints in nephropathy trials. Cystatin C is a 13 kDa cysteine protease that is ubiquitously expressed and released into blood. Serum cystatin C (S-cystatin C) is freely filtered by the glomerulus and is age, gender and muscle mass independent. S-cystatin C and S-creatinine are equally accurate in estimating GFR, according to accepted industry and academic guidance [33], yet there are circumstances in which S-cystatin C offers clear added value over S-creatinine for GFR determination. Patients with small muscle mass (e.g. some women or children) show reduced creatinine production, making S-cystatin C GFR determinations preferred [33]. Combining both formulas (S-cystatin C and S-creatinine) provides greater accuracy, but experience with S-cystatin C measurements and GFR determination is recommended and adds greater cost to the analysis [33]. S-cystatin C is superior to S-creatinine in estimating ^{51}Cr-EDTA clearance (GFR) with nonage-adjusted diabetics, but no difference was found with age adjustment [34]. Age adjustment addresses pathology from renal disease compared with renal decline from normal aging. Diagnostic accuracy in receiver–operator curve analysis showed S-cystatin C to have a greater area under the curve (AUC) (0.93) than S-creatinine (0.68) at a GFR 80 mL min^{-1} 1.73 m^{-2} cutoff, but no difference at a lower GFR cutoff [27,34]. Thus, S-cystatin C is a better assessment of patients early in their disease progression and is recommended as an improved efficacy marker for application in clinical trials. Serum β2-microglobulin and cystatin C show a greater dynamic range for GFR determinations than creatinine and are gender and age independent, which is an advantage for clinical trial recruitment. Whether β2-microglobulin determinations show advantages in normoalbumin patients has not been studied but is anticipated. Hence, S-cystatin C offers clear superiority in detecting mild nephropathy (CKD stages 1 and 2), which adds great value in clinical trials in which the number of subjects is limited and recruitment specifications are complex. Note that urinary cystatin C was qualified for renal injury in the VXDS, not S-cystatin C, although its application is recommended [1,2].

Concluding remarks

Whereas albumin is an excellent marker for nephropathy decision-making in clinical trials, additional biomarker endpoints are recommended to fill the albumin gap in 25% of patients with normo-levels. S-cystatin C provides superior GFR determinations in the patients with a defined albuminuria gap, and CB elevations provide another potential marker documented to address the gap. Whether novel renal injury biomarkers would show added value to albumin in this clinical model of diabetic nephropathy has not yet been addressed but is anticipated in future investigations, perhaps with existing archived samples. Proximal tubular enzymes CB and NAG are recommended for evaluation in the lead optimization paradigm as anchors in addition to the commercially available MSD and Luminex renal toxicity biomarkers.

Note added in proof

The PSTC is sponsoring a special *Nature Biotechnology* issue on the VXDS renal biomarker submission to the FDA and EMEA. Four research manuscripts and additional commentaries for this issue are *in press* and support [1,2] as additional citations for this paper including: Ozer et al., Urinary biomarkers to monitor reversibility of renal injury whereas serum cystatin C detects functional capacity; Dieterle et al, Qualification of urinary clusterin to detect drug-induced tubular injury and of urinary cystatin C, β2-microglobulin and total protein to detect drug-induced glomerular injury; Yu et al., Biomarkers of Kidney Tubule Injury: Urinary Trefoil Factor 3 and Albumin; Vaidya et al., Kidney Injury Molecule-1 Outperforms Traditional Biomarkers of Kidney Injury in Multi-site Preclinical Biomarker Qualification Studies; Sistare et al., Towards Establishing Consensus Qualification Practices for New Safety Biomarkers in Early Drug Development and Regulatory Decision-Making; and Dieterle et al., Renal Biomarker Qualification Submission: A dialog between the FDA/EMEA and PSTC.

Acknowledgements

I thank Tim Heath, Sven Beushausen, Denise Robinson-Gravatt, and Frank Sistare for reading the manuscript and providing helpful suggestions. Dominic Eisinger and I shared stimulating conversations on this topic. Leonid Kirkovsky and Anup Zutshi made helpful comments on the concept for the article.

References

1 EMEA (2008) Committee for human medicinal products, final report on the pilot joint EMEA/FDA vxds experience on qualification of nephrotoxicity biomarkers. http://www.emea.europa.eu/pdfs/human/biomarkers/25088508en.pdf
2 FDA (2008) FDA, European Medicines Agency to consider additional test results When assessing new drug safety collaborative effort by FDA and EMEA expected to yield additional safety data. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2008/ucm116911.htm
3 Perico, N. *et al.* (1992) Daily renal hypoperfusion induced by cyclosporine in patients with renal transplantation. *Transplantation* 54, 56–60
4 Pollard, S. *et al.* (2003) A pharmacokinetic and clinical review of the potential clinical impact of using different formulations of cyclosporin A. *Clin. Ther.* 25, 1654–1669
5 Kasiske, B.L. *et al.* (1988) The relationship between cyclosporine pharmacokinetic parameters and subsequent acute rejection in renal transplant recipients. *Transplantation* 46, 716–722
6 Kopp, J.B. and Klotman, P.E. (1990) Cellular and molecular mechanisms of cyclosporin nephrotoxicity. *J. Am. Soc. Nephrol.* 1, 162–179
7 Myers, B.D. *et al.* (1984) Cyclosporine-associated chronic nephropathy. *N. Engl. J. Med.* 311, 699–705
8 Lafayette, R.A. *et al.* (1993) The effects of blood pressure reduction on cyclosporine nephrotoxicity in the rat. *J. Am. Soc. Nephrol.* 3, 1892–1899
9 Ptachcinski, R.J. *et al.* (1986) Clinical pharmacokinetics of cyclosporin. *Clin. Pharmacokinet.* 11, 107–132
10 Aleo, M.D. *et al.* (2008) An underlying role for hepatobiliary dysfunction in cyclosporine A nephrotoxicity. *Toxicol. Appl. Pharmacol.* 230, 126–134
11 Badr, K.F. *et al.* (1984) Renal and systemic hemodynamic responses to intravenous infusion of leukotriene C4 in the rat. *Circ. Res.* 54, 492–499
12 Perico, N. *et al.* (1991) Co-participation of thromboxane A2 and leukotriene C4 and D4 in mediating cyclosporine-induced acute renal failure. *Transplantation* 52, 873–878
13 Butterly, D.W. *et al.* (2000) A role for leukotrienes in cyclosporine nephrotoxicity. *Kidney Int.* 57, 2586–2593
14 Benigni, A. *et al.* (1988) Increased urinary excretion of thromboxane B2 and 2,3-dinor-TxB2 in cyclosporin A nephrotoxicity. *Kidney Int.* 34, 164–174
15 Duane, W.C. *et al.* (1976) Effects of fasting on bile acid metabolism and biliary lipid composition in man. *J. Lipid Res.* 17, 211–219
16 Huber, M. *et al.* (1990) Metabolism of cysteinyl leukotrienes in monkey and man. *Eur. J. Biochem.* 194, 309–315
17 Laidlaw, S.A. *et al.* (1994) Patterns of fasting plasma amino acid levels in chronic renal insufficiency: results from the feasibility phase of the Modification of Diet in Renal Disease Study. *Am. J. Kidney Dis.* 23, 504–513
18 Tucholska, M. *et al.* (2009) Human serum proteins fractionated by preparative partition chromatography prior to LC–ESI-MS/MS. *J. Proteome Res.* 8, 1143–1155
19 Kaplowitz, N. (2005) Idiosyncratic drug hepatotoxicity. *Nat. Rev. Drug Discov.* 4, 489–499
20 Lee, W.M. (2003) Drug-induced hepatotoxicity. *N. Engl. J. Med.* 349, 474–485
21 Basi, S. *et al.* (2008) Microalbuminuria in type 2 diabetes and hypertension: a marker, treatment target, or innocent bystander? *Diabetes Care* 31 (Suppl. 2), S194–S201
22 Schiffrin, E.L. *et al.* (2007) Chronic kidney disease: effects on the cardiovascular system. *Circulation* 116, 85–97
23 Meinhardt, U. *et al.* (2003) Microalbuminuria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collection. *J. Diabetes Complications* 17, 254–257
24 Ruggenenti, P. and Remuzzi, G. (2006) Time to abandon microalbuminuria? *Kidney Int.* 70, 1214–1222
25 Atkins, R.C. *et al.* (2005) Proteinuria reduction and progression to renal failure in patients with type 2 diabetes mellitus and overt nephropathy. *Am. J. Kidney Dis.* 45, 281–287
26 de Zeeuw, D. *et al.* (2004) Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int.* 65, 2309–2320
27 Jerums, G. *et al.* (2008) New and old markers of progression of diabetic nephropathy. *Diabetes Res. Clin. Pract.* 82 (Suppl. 1), S30–S37
28 Tsalamandris, C. *et al.* (1994) Progressive decline in renal function in diabetic patients with and without albuminuria. *Diabetes* 43, 649–655
29 Rigalleau, V. *et al.* (2007) Normoalbuminuric renal-insufficient diabetic patients: a lower-risk group. *Diabetes Care* 30, 2034–2039
30 Schaefer, L. *et al.* (1994) Urinary excretion of cathepsin B and cystatins as parameters of tubular damage. *Kidney Int. Suppl.* 47, S64–S67
31 Piwowar, A. *et al.* (2006) Urinary activities of cathepsin B, *N*-acetyl-beta-D-glucosaminidase, and albuminuria in patients with type 2 diabetes mellitus. *Med. Sci. Monit.* 12, CR210–CR214
32 Salem, M.A. *et al.* (2002) Urinary excretion of *n*-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. *Pediatr. Diabetes* 3, 37–41
33 Tidman, M. *et al.* (2008) A comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two. *Nephrol. Dial. Transplant.* 23, 154–160
34 Christensson, A.G. *et al.* (2004) Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. *J. Intern. Med.* 256, 510–518
35 Ozer, J.S. and Teitelbaum, A. (2009) Novel prodromal biomarkers that are symptomatic of injury require histopathologic or clinical chemistry anchors to characterize activity. *J. Pharmacol. Toxicol. Methods* 60, 321–322