BIOMARKER QUALIFICATION PROGAM
OFFICE OF CLINICAL PHARMACOLOGY
FULL QUALIFICATION PACKAGE REVIEW

Application	FNIH/PSTC PFC Index Biomarker Qualification
Submission Date	08/06/2015
Submitter	FNIH/PSTC
BQ Scientific Lead Reviewer(s)	Martina Sahre, Ph.D., Katarzyna Drozda, Pharm.D., M.S.
BQ Program Lead	Hobart Rogers, Pharm.D., Ph.D.

EXECUTIVE SUMMARY

The Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium Kidney Safety Biomarker Project Team and the Critical Path Institute's (C-Path) Predictive Safety Testing Consortium Nephrotoxicity Working Group (FNIH BC/PSTC NWG) have jointly submitted a full qualification package for a composite safety biomarker of renal tubular injury response to be used in normal health volunteer (NHV) trials during early drug development (herein referred to as the PFC (PSTC/FNIH/C-Path) index). The composite measure consists of the following individual biomarkers: clusterin (CLU), cystatin-C (CysC), kidney injury molecule-1 (KIM-1), N-acetyl-beta-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), and osteopontin (OPN). In the context of a nonclinical nephrotoxicity signal, group-level increases in the index above a threshold determined by individual sponsors would indicate the potential presence of renal tubular injury.

The submitter's primary support for the proposed Context of Use (COU) is a study of 76 normal health volunteers (NHVs) from whom data were collected at baseline and post-baseline on day 20 (±2). From this study the submitter developed a composite measure as the PFC index. Subjects in the NHV study did not receive a pharmacological intervention. The study was intended to assess "normal" biomarker levels in a NHV population. The submitter also utilized data from cisplatin-treated mesothelioma patients in an attempt to validate the PFC index in patients who are receiving a known nephrotoxicant. The submitter's NHV dataset has limitations insofar as the following are not addressed: intra-subject variability of the individual biomarkers, time courses of the individual biomarkers in relation to sample collection, and intrinsic factors that could affect biomarkers at baseline and the response of each biomarker to a nephrotoxicant (e.g. age, sex, or race). Additionally, the mesothelioma study was conducted in patients who are not NHVs, so it adds little to support the proposed COU (which is focused on early phase NHV studies in drug development). However, it does help by offering insight into potential PFC index differences between NHVs and a population different from NHVs.

Despite the limitations of what is known about the biomarkers, the submitter's PFC index may add value to traditional measures of renal tubular injury, allowing sponsors to further assess the likelihood of renal tubular injury in phase 1 studies, and it could foster the willingness of sponsors to measure these novel markers in cases where nephrotoxicity is suspected from preclinical studies. In addition to the PFC index, individually, each of the six biomarkers may add value when their standalone values in the dosing cohort differ from the NHV population.

Because the merits outweigh the limitations, it is the opinion of the Office of Clinical Pharmacology that the submitter's PFC index can be qualified as a safety biomarker of renal tubular injury to be used optionally in conjunction with standard measures of renal function (serum creatinine [sCr], blood urea nitrogen [BUN], urine albumin, and urine total protein) to monitor the risk of renal tubular injury in NHV phase 1 clinical trials. Moreover, it is also the opinion of the Office that increases in any of the individual urine biomarkers in the PFC index beyond the range identified in the NHVs may also provide evidence of renal tubular injury. The opinion of the Office is dependent on the determination by CDRH that the submitter and subsequent users of the PFC have analytical methods that allow the measurement of each individual marker with robustness, sensitivity, and specificity as outlined in relevant Guidance to Industry. If that cannot be achieved, then the applicability of the NHV data derived PFC thresholds will be questionable. Additionally, the fact that a threshold will have to be determined in a product-specific way as proposed by the submitter may limit the generalizability of a given threshold across molecules.

1 BACKGROUND

The current standard safety biomarkers used to monitor kidney function and toxicity are sCr, BUN and urinary protein. A commonly cited weakness of sCr is that changes in this marker typically occur late (24-48 h post-insult) and can depend on baseline renal function and renal reserve of a particular patient.[1] The American College of Radiology Committee on Drugs and Contrast Media estimates that normal serum creatinine is maintained until the creatinine clearance is reduced by about 50%.[2] Currently, no biomarkers are qualified for use in clinical studies to identify the presence of drug-induced renal injury. Renal papillary antigen-1, KIM-1, albumin, total protein, β2-microglobulin, cystatin C, clusterin and Trefoil factor-3 are qualified for use in animal toxicology studies to detect acute drug-induced renal tubule alterations. According to the submitter, all of these individual biomarkers have been correlated with subtle histomorphologic kidney damage in preclinical studies. Exploratory investigations in a variety of clinical settings have demonstrated that elevations in the levels of these biomarkers can be observed even prior to sCr elevation and provide an early signal of renal tubular injury, as referenced in Table 1.

The submission reviewed herein is a full qualification package from the FNIH BC/PSTC NWG (hereafter referred to as the submitter) for a composite biomarker composed of six urine proteins, including CLU, CysC, KIM-1, NAG, NGAL, and OPN. The proposed context of use is as follows: a composite measure (PFC index) of urine CLU, CysC, KIM-1, NAG, NGAL, and OPN to be qualified as a safety biomarker of kidney tubular injury response for use in NHV trials during early drug development. The submitter proposes to use the PFC index in NHVs in single or multiple ascending dose phase 1 trials when preclinical animal toxicology studies have shown elevations in renal markers of tubular injury. The PFC index obtained in a NHV study would be

[1] Zuk A, Bonventre JV. "Acute Kidney Injury" Annu Rev Med 2016;67:293
[2] ACR Committee on Drugs and Contrast Media "ACR Manual on Contrast Media, Version 10.1" (2015), page 34, cited 5/24/2016, available from: http://www.acr.org/Quality-Safety/Resources/Contrast-Manual

compared to a threshold that would be set based on the risk-tolerance associated with the therapeutic indication under investigation. Timing of sample collection should be informed by animal toxicology study data for the study drug. The proposed PFC index is intended to complement the standard measures used for kidney safety monitoring (e.g., sCr, BUN, urine albumin and urine total protein) and is not intended for individual patient safety monitoring. An increase in the geometric mean of the PFC index above a particular threshold is intended to be an indicator that the new drug or a given dose is potentially nephrotoxic and might be considered to be potentially unsafe. In this case, a potential decision to stop the study or treatment arm should be considered, along with further investigation as to the cause of the increase in threshold values.

The primary data to support qualification for this proposed context of use were obtained from NHVs. The PFC index was derived using the fold change from baseline in urine biomarker data by three different methods: a principal component analysis (PCA), a timepoint-specific group average PFC index (calculated as a geometric mean [GM]) and the longitudinal equal weighted PFC index.

The purpose of this review is to determine if the submitted evidence is suitable to provide support for the qualification of the proposed biomarkers under this COU.

Table 1 provides an overview of the proposed biomarkers based on the submitter's materials and review of literature.

Table 1. Characteristics of Urinary Biomarkers of Nephrotoxicity

Urinary Biomarker	General Characteristics	Mechanism of Injury
Clusterin (CLU)	• Product of the *CLU* gene[1] • Expressed in human endometrium in a hormone dependent manner[1] • Expressed at high levels during renal development and in response to kidney injury in the proximal and distal tubules, glomerulus, and collecting duct[1]	• CLU has been suggested to play an anti-apoptotic role and be involved in cell protection, lipid recycling, cell aggregation and cell attachment[1]
Cystatin-C (CysC)	• Product of the *CST3* gene[1] • Freely filtered at the glomerulus and then reabsorbed by the renal tubular epithelium[1] • Normal uCysC concentration below 0.3mg/L[1] • Short plasma $t_{1/2}$ (20 min in mice, 2 hours in humans)[3] • uCysC levels are increased in: systemic inflammation, treatment with high glucocorticoid doses, and hyperthyroidism[6]	• Serum CysC is a sensitive marker of kidney function (GFR)[6]

Urinary Biomarker	General Characteristics	Mechanism of Injury
Kidney Injury Molecule-1 (KIM-1)	• Product of the *TIM-1/HAVCR-1* gene[1] • Not expressed in normal kidney[6] • Anoxia and ischemia to the kidney have been reported to stimulate gene expression and urinary concentration[1] • The normal range for KIM-1 in healthy volunteers is 0.1 to 1.2 ng/mg Cr, whereas a threshold of 1.6 ng/mg Cr has been proposed as indicative of AKI[1]	• KIM-1 mRNA levels are elevated after initiation of kidney injury more than any other known gene[1] • Marker of AKI at12-24 hours[6]
N-Acetyl-beta-D-Glucosaminidase (NAG)	• Product of the *Nag1* gene[1] • Lysosomal brush-border enzyme with two isoforms (A and B) expressed in proximal tubule[1] • Activity inhibited by endogenous urea, nephrotoxicants and heavy metals[2] • uNAG levels are increased in: rheumatoid arthritis, impaired glucose tolerance, and hyperthyroidism[2]	• Subtle alterations in the epithelial cells in the brush border of the proximal tubules result in shedding of NAG with direct correlation of the amount to tubular injury[2] • Precedes increases in sCr by 12 hours to 4 days in a population of critically ill adult patients[5]
Neutrophil gelatinase-associated lipocalin (NGAL)	• Product of the *LCN2* gene[1] • Expressed in various tissues at low levels, but induced in epithelial cells with inflammation, infection and malignancy[1,5]	• Rapidly upregulated during kidney injury in the thick ascending limb of the loop of Henle, distal tubule and collecting duct, and is secreted into the urine and plasma[1] • Marker of AKI at 2-6 hours[6] • An early indicator of AKI following cisplatin administration[5] • Precedes increase in sCr by 1-3 days[2]
Osteopontin (OPN)	• Product of the *SPP1* gene[1] • Synthesized at the highest levels in bone and epithelial tissues[2] • Protective agent against oxidative stress and ischemia, has pro-inflammatory and profibrotic activity[1] • In normal kidney expressed at low levels in the distal nephron (thick ascending limb of the loop of Henle and distal convoluted tubules)[1]	• During tissue injury, increased expression has been demonstrated throughout the kidney[1] • Sensitive and inducible indicator of different forms of AKI[1]

Source: [1]Submitter's Briefing Book; [2]PMID: 17937594; [3]PMID: 16519600; [4]PMID: 16174863; [5]PMID: 18294749; [6]Edelstein, CL. Biomarker of kidney disease (2011).

2 SUBMISSION CONTENTS

Data from two studies were submitted, a NHV study and a study in mesothelioma patients treated with cisplatin, in order to support the use of the PFC index as a qualified safety biomarker of kidney tubular injury response.

NHV Study

The study titled "An Observational Biomarker Study to Characterize the Intra- and Inter-Subject Variability in Healthy Volunteers" was performed between May 2011 and October 2012 and is the primary evidence submitted to support the proposed COU.

The primary study objectives were as follows:

- To characterize the mean values, normal range and inter- and intra-subject variability of renal biomarkers (including, but not limited to, urine CLU, OPN, NAG, KIM-1, CysC, and NGAL) in healthy subjects.
- To assess whether the mean values or variability are influenced by age, sex, or time of day.

The secondary study objectives were as follows:

- To create a well-annotated sample set for evaluation of other biomarkers submitted by the PSTC in the future.
- To establish assay performance criteria.
- To collect blood for future exploratory studies correlating genomic patterns with biomarker expression.

Reviewer's Note: One of the sponsor's stated objectives is whether the mean values of biomarkers are dependent on time of day. It should be noted that the study design does not include multiple sample collections per study day. Therefore, this assessment cannot be made from the study data. Furthermore, within-subject variability of the biomarkers is assessed from only two time points (20 days apart), for most of the biomarkers. The sponsors should assess this variability over a shorter time span as well, and with more than two measurements to account for acute as well as long-term changes in the marker that are likely physiological and not cause for toxicity concern.

The study inclusion criteria were as follows: male and female subjects of age 18-70 years; no underlying diagnosis or use of chronic medications or creatinine supplements; sitting systolic blood pressure of <140 mmHg and >90 mmHg; sitting diastolic blood pressure >40 mmHg and <90 mmHg; creatinine clearance (Cockcroft-Gault method) of >90 mL/min/1.73m^2 (for age group 18-39 years), and creatinine clearance of >75 mL/min/1.73m^2 (for age group 40-70 years); body mass index (BMI) <35 (with some exceptions).

The study exclusion criteria were as follows: HIV, Hepatitis B or C; a medical intervention ≤3 months prior to study enrollment; self-reported urinary tract infections ≤6 months; confirmed abnormal thyroid function; unwilling to refrain from illicit drug, alcohol, or tobacco use or strenuous exercise during the study period; female subjects with a positive urine pregnancy test.

Each subject provided a morning void spot urine collection and blood sample for analysis of sCr and biomarker levels at the clinic visit 1 (day 1; CLU, OPN, NAG, KIM-1, CysC, and NGAL), visit 3 (day 6±1; only CLU and NAG) and visit 4 (day 20±2; CLU, OPN, NAG, KIM-1, CysC, and NGAL). All urine biomarkers samples were normalized to urinary creatinine (uCr) level in order to approximate urinary biomarker excretion rate or absolute biomarker concentration in the setting of onset of acute kidney injury (AKI). Urinary biomarkers are often normalized as mentioned above to account for differences in urinary flow rate.

CLU, CysC, KIM-1, NGAL, and OPN were analyzed using commercially available ELISA assays; the analytical method for NAG utilized a colorimetric detection method. Creatinine was measured using an enzymatic assay. Except for creatinine, all assays were labeled "For Research Use Only" (i.e. they have not been cleared by FDA) however, they are compliant with methodology used by College of American Pathologists (CAP).

Reviewer's Note: No pharmacologic interventions were given to elicit renal tubular injury in the NHV study.

Mesothelioma Study

In addition to NHV data submission, results for patients in a Mesothelioma Study (Meso study) were also provided. In this study, urine samples were collected in a cohort of patients who underwent surgical resection (extrapleural pneumonectomy) for the treatment of malignant pleural mesothelioma and were treated with intraoperative intrathoracic cisplatin 250 mg/m^2. Urine and serum were collected prior to surgery and cisplatin treatment, and over a period of up to 6 days following cisplatin treatment. Biomarker data were collected from 39 patients without evidence of chronic kidney disease (CKD) at baseline. The Mesothelioma Study consisted of 3 subgroups: Meso Surgery (n=4, surgical control patients without exposure to cisplatin), Meso Controls (n=22, patients exposed to cisplatin without renal injury [i.e., increases in sCr ≤50% and ≤0.3 mg/dL above baseline]), and Meso Cases (n=13, patients exposed to cisplatin with renal injury [i.e., increases in sCr >50% and/or >0.3 mg/dL above baseline]). The obtained PFC index values for the mesothelioma subgroups were used to assess the performance of the PFC index in patients with known exposure to the nephrotoxicant cisplatin and AKI.

3 RESULTS

Review of Submitted Data/Results: NHV Study

Eighty nine NHVs were eligible for study enrollment. A total of 81 subjects completed the study (eight subjects prematurely discontinued voluntarily or due to protocol violations). The data used to derive the PFC index and define normal variability PFC index thresholds were obtained from 76 NHVs with all six biomarkers at both visits (visit 1 and 4). Baseline subject demographics are provided in Table 2 below.

Table 2. Demographics at Study Entry for PSTC NHV Study (Patients Who Completed the Study with Biomarker Data)

	Age Category		
	20 – 39 years N=41	40 – 70 years N=40	Total N=81
Age (years)			
Mean	29.4	50.8	40.0
Median	30.0	50.5	39.0
Min, Max	20.0, 39.0	40.0, 69.0	20.0, 69.0
Sex, n (%)			
Male	20 (48.8%)	20 (50.0%)	40 (49.4%)
Female	21 (51.2%)	20 (50.0%)	41 (50.6%)
Race, n (%)			
White	32 (78.0%)	36 (90.0%)	68 (84.0%)
Non-white	9 (22.0%)	4 (10.0%)	13 (16.0%)
Ethnicity, n (%)			
Hispanic	1 (2.4%)	1 (2.5%)	2 (2.5%)
Non-Hispanic	40 (97.6%)	39 (97.5%)	79 (97.5%)
BMI (lb/in^2)*			
Mean	26.8	28.0	27.4
Median	27.76	27.89	27.76
Min, Max	19.1, 35.4	20.4, 37.6	19.1, 37.6
CDC BMI Category, n (%)			
Normal	18 (43.9%)	7 (17.5%)	25 (30.9%)
Overweight	10 (24.4%)	19 (47.5%)	29 (35.8%)
Obese	13 (31.7%)	14 (35.0%)	27 (33.3%)

BMI = body mass index; CDC = Centers for Disease Control and Prevention; in = inches; lb = pounds; max = maximum; min = minimum
** - Subjects with BMI greater than 35 were included in the study.*

Source: Submitter's Briefing Book

The observed ranges for the biomarkers in the NHV population were supplied in the submitter's response document from September 2, 2015 and are found in Table 3 below.

Reviewer Note:
Please refer to the review of the analytical methods by CDRH (Reviewer: Kellie Kelm, Date: 03/28/2016), for further details. Briefly, there is no in-use validation of the performance of the analytical tests. This will need to be resolved prior to qualification of the test.

Table 3. Observed Range of Biomarker Concentrations in the NHV Study, Assay Range

Biomarker	***Total Population GM (LLN, ULN)***	***Female***	***Male***	***18-39 y***	***40-60 y***	***Assay Range***
OPN (µg/dL)	104.7 (10.8, 1015.8)	98.0	111.4	133.2	82.0	4-25600
CLU (ng/dL)	10329.2 (1235.2, 86379.4)	11310.4	9392.2	12745.6	8334.6	1000-320000
CysC (µ g/dL)	2.50 (0.18, 35.4)	2.37	2.63	3.26	1.91	0.14-640
KIM-1 (ng/dL)	36.1 (2.8, 469)	37.1	35.0	37.7	34.4	1.2-6400
NAG (mU/dL)	141.1 (19, 1048.4)	135.7	146.6	155.8	127.7	31-221000
NGAL (ng/dL)	1168.1 (60.1, 22686.4)	1988.2	673.4	1449.4	923.8	40-640000

Source: Reproduced from Table 1A and 2, from FNIH Response Document from 2 September 2015 (Assay Validation Response.pdf)

The geometric mean fold-change from baseline biomarker concentrations in the NHV population is shown in Table 4. Of note, the coefficients of variation ranged from 43 to 70%, indicating substantial variability in the data.

Reviewer Note:
Without intervention, geometric mean fold-changes for the biomarkers ranged from -5 to 12% over a time span of 20 days. All 95% confidence intervals hovered close to 1, i.e. there was no clinically significant fold-change increase in these particular subjects over the 20-day time period.

Table 4. Geometric Mean Changes from Baseline Biomarker Values in the NHV study (Day 20)

Biomarker	Geometric Mean (%CV)	95% interval on GM FC
CLU	1.00 (56.8)	0.88, 1.12
OPN	1.12 (53.3)	1.00, 1.26
NAG	0.95 (50.1)	0.85, 1.06
KIM-1	1.07 (43.4)	0.98, 1.18
CysC	1.11 (50.0)	1.00, 1.24
NGAL	1.11 (69.6)	0.96, 1.28

Source: Table 12 in Submitter's Briefing Book (KSP_FNIH_PSTC_CoUBriefingBook_FINAL_2015-07-27.pdf)

Tables 5 and 6 show the within- and between-subject coefficient of variation (CVw and CVb, respectively) for the geometric means biomarker measurements. Based on two measurements (three for NAG and CLU), CVw ranged from 96% for NAG to 136% for cystatin C (non-normalized for urine creatinine) and from 31% for KIM-1 to 47% for NGAL for the urinary creatinine normalized values. *CVb tended to be less than CVw.*

Table 5. Geometric mean (GM), within-subject (CVw), between-subject (CVb) and total (CVtot) coefficient of variation of GM in the NHV study (non-normalized values)

Biomarker	GM	CVb	CVw	CVtot
OPN	104.7	85.5	110.2	168.3
CLU	10329.2	72.9	105.5	149.5
CYSC	2.50	108.4	136.4	228.5
KIM-1	36.1	122.7	110.2	213.2
NAG	141.1	69.2	96.3	136.0
NGAL	1168.1	163.3	130.2	298.0
Albumin	370.6	77.8	64.8	113.2
Total Protein	5.16	40.9	51.5	69.0

Source: DraftNormalHealthyVolunteerManuscript.pdf, Table 5 A.
Index Ind. Refers to a comparison between CVb and CVw.

Table 6. Geometric mean (GM), within-subject (CVw), between-subject (CVb) and total (CVtot) coefficient of variation of GM in the NHV study (urine creatinine normalized values)

Biomarker	GM	CVb	CVw	CVtot
OPN	0.980	38.1	36.3	54.4
CLU	109.1	48.2	43.0	67.8
CYSC	0.023	36.7	35.8	53.0
KIM-1	0.337	77.3	30.5	86.4
NAG	1.50	31.3	38.1	50.7
NGAL	10.9	121.1	47.4	142.2
Albumin	3.47	89.9	63.9	124.4
Total Protein	0.048	54.9	50.1	79.2

Source: DraftNormalHealthyVolunteerManuscript.pdf, Table 5 B.

Reviewer Comment: Given that the ranges of the biomarker were developed from this small dataset from NHV, and the relatively large between- and within-subject variability, it is unclear whether the variability observed here really describes the expected variability of the biomarker in the population well enough. Issues may arise with normal healthy volunteer populations who have different ranges at baseline or different fluctuations over a given time period. In such a case, the utility of the PFC index threshold would be uncertain.

Calculation and Use of the PFC Index (referred to as the CM below)

The equal weighted composite measure is calculated as follows:

$$CM_{\text{Subject i, Time t}} = \exp[(1/6)*\ln(FC_{CLU,i}) + (1/6)*\ln(FC_{OPN,i}) + (1/6)*\ln(FC_{NAG,i}) + (1/6)*\ln(FC_{KIM-1,i}) + (1/6)*\ln(FC_{NGAL,i}) + (1/6)*\ln(FC_{CysC,i})]$$

where FC is a single individual's fold change from baseline and *i* refers to the individual subject.

The geometric mean PFC index of a particular cohort, i.e. study drug or placebo is then calculated as follows:

$$\text{GM CM for one Group} = \exp(\sum_{i=1}^{n} \ln(\text{CM}_i)/n)$$

Reviewer Comment: From the NHV population, the submitter developed a threshold referring to multiple sample sizes per group and probabilities for observing a result equal to or greater than the threshold. Please refer to the Statistical Review by Dr. Soukup for a thorough review of the development of these thresholds.

The PFC index is intended to be used by a sponsor with an NME in a SAD/MAD study in phase 1. After calculating the observed geometric mean (GM) of the PFC index, it is then compared to the threshold value. The sponsor chooses the probability for the threshold based on information about the study drug, the tolerance for risk for a particular disease, and potentially other factors. For example, if a sponsor chose to study 6 subjects on active treatment in a single dose arm in the first-in-human study, and was comfortable with a 5% probability of overlapping with the NHV population, then the threshold would be 1.27 (Table 1 in Submitter's Briefing Book). Meeting or exceeding the threshold essentially means that the cohort that has been studied at a particular dose is not consistent with the NHV population from which the thresholds were derived, and that the probability of observing a GM of the PFC index as large as or greater than the threshold in a NHV population is less than 5%.

Validation in the Meso Study

The submitter provided an additional data set from a cohort of 116 mesothelioma patients treated with cisplatin. The purpose of this data set was to evaluate the performance of the PFC index derived from the NHV study. From this cohort, samples of the 6 urine biomarkers were available for 58 subjects at baseline and up to nine timepoints after dosing. Data from the mesothelioma study indicated that patients had elevated biomarker concentrations at baseline. The GM of the PFC index for the group of patients receiving cisplatin treatment and being classified as having developed AKI (>50% increase or >0.3 mg/dL increase post-baseline sCr) (Meso Cases), the patients on cisplatin who did not develop AKI (Meso Controls), and patients who were treated with surgery instead of cisplatin (Meso surgery) all showed values above the NHV threshold at all time-points post-baseline.

Key Considerations for Use of the Proposed Biomarker

Time Courses of PFC Index Biomarkers in Relation to Renal Tubular Injury
The time course of excursions of some of the biomarkers in the PFC index as a result of renal tubular injury is unclear. The submitter has collected samples at three time points throughout the NHV study, but only analyzed samples from baseline and 20 days post-treatment, except for

NAG and CLU, where all three samples were analyzed. Samples obtained on day 6 were not analyzed, and it is not clear if these samples, even with freezer storage, are still viable for analysis. Samples were collected at first clinic visit with no reference to time of day, hence one of the study objectives of accounting for variation by time of day are not met. As a result there are several questions unanswered which may be relevant to determine optimal sampling times, as follows:

- What is the time course of the individual markers in relation to insult?
- What is the natural fluctuation of the individual markers, without insult?
- When, relative to each other, does each biomarker show excursions that are sensitive and specific enough to be counted as indicative of renal tubular injury?

As a condition of use, the submitter states that sample collection times should be informed by animal toxicology study data for the study drug. This can be problematic in that the time course of the biomarkers following renal injury might be different from one another. For example, following ischemic injury during open heart surgery, the time courses of KIM-1, NAG, and NGAL are different (Figure 1).Therefore, an optimal sampling scheme for these markers does, so far, not exist. Sampling without regard to the kinetics of each biomarker raises the risk of missing elevations, which could result in false negatives.

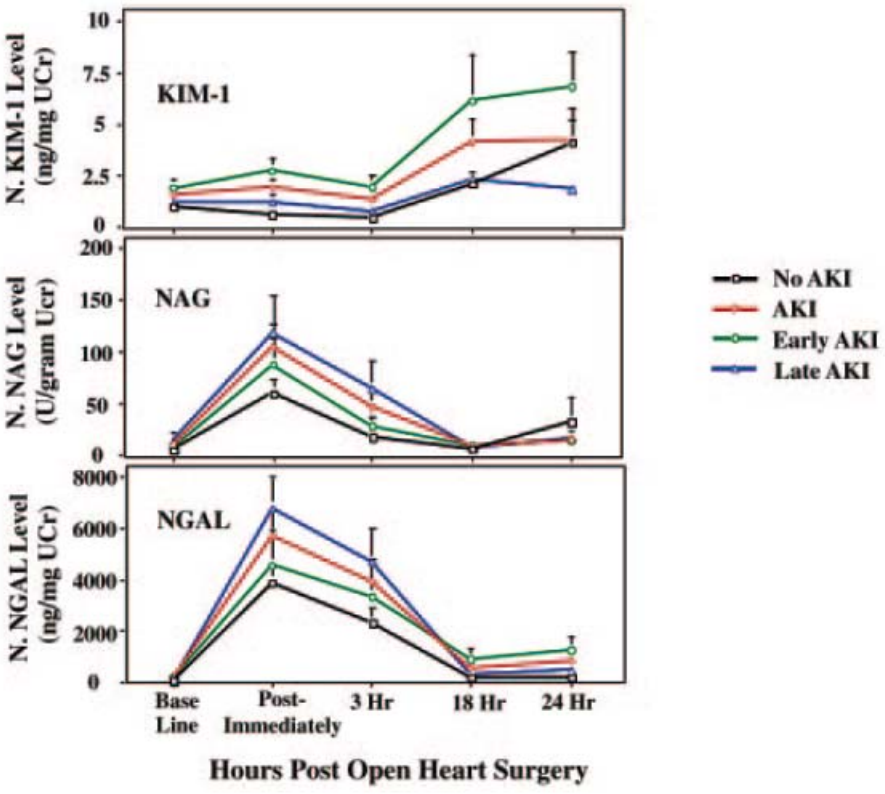

Figure 1. Pattern of Urinary Biomarker Expression Following Cardiac Surgery
Source: Han et al., Clin J Am Soc Nephrol 2009;4: 873

Intrinsic Factors as Covariates for PFC index Biomarker Concentrations
The submitter currently does not consider race, age, or sex as a factor in their index, but these covariates can influence the concentrations of the biomarkers in the PFC index (Table 2).[3] This issue was addressed during a teleconference, where the submitter mentioned that differences in biomarker levels that were observed between male and female study participants did not affect the PFC index. This finding may be attributable to the small sample size of the NHV study. The internal discussion centered on the problems this could create if other studies do not enroll similar numbers of males and females or different age cohorts compared to the NHV study used to define the thresholds. This type of analysis could be done once more data from several studies becomes available.

Conditions Where the Biomarkers May Not Perform Well
The PFC index measure is not intended for use in cohorts with baseline values outside of the expected ranges. As demonstrated by the Mesothelioma study, validation of the established in NHV thresholds would not provide efficient assurance for use of the PFC index biomarkers. This observation was expected since the developed thresholds are intended to be applied only in phase 1 studies of NHVs. It is also important to recognize that if a future study population differs from that of the NHV study with respect to factors that may influence the performance of the biomarker (e.g., age, weight, sampling times) different thresholds may be needed or interpretation of data might be compromised.

Selection of Biomarkers: The submitter does not go into detail about the selection of the particular biomarker compared to other potential biomarkers (e.g. TIMP-2 or IGFPB7) that have been associated with some degree of being able to correlate with renal tubular injury.[4]

4 SUMMARY AND CONCLUSIONS

In summary, there are a number of limitations that make the potential use of the PFC difficult.

1. The dataset use to derive the thresholds is small (NHV dataset n=76), which limits information about sources of variability that likely exist in the general healthy population (i.e. based on sex, race, or age).
2. Intra-subject variability in the time-course of each biomarker is not well assessed (only two samples over a 20 day time span in most subjects were collected).
3. There was no assessment on the potential for diurnal variations in biomarker concentrations in urine. This knowledge would be an important factor for the determination of optional sampling times.
4. The time course of the biomarkers following renal tubular injury was not assessed (there was no intervention with a potential nephrotoxicant in the NHV study), and data in the medical literature are relatively scarce. As suggested by the submitter, the optimal urine biomarker sampling time is to be derived from animal toxicology studies, but that

[3] DraftNormalHealthyVolunteerManuscript.pdf (submitted 11/23/2015 in response to an Information Request by FDA)
[4] PMID: 26948834, PMID 24675717

assumes that there is sufficient overlap between preclinical species and humans with regard to the time course of biomarker excursions from normal. This assumption has, to the knowledge of the reviewer, not been systematically assessed. The accuracy of biomarker time course and how closely the urine sampling time adheres to it are important because of the risk for false-negative findings.

5. There was no separate information from a study in NHV where a known to cause renal tubular injury was administered. Therefore, the performance of the PFC index in the situation for which it was developed has, so far, not been shown. The submitters are currently conducting such a study, but data was not available for this review.

While the PFC index is currently supposed to be used on a cohorts level, more than likely, review of results would also assess individual biomarker time courses in an individual subject. Significant increases in individual urine biomarkers in the PFC index may provide evidence of renal tubular injury, but, the caveat here is that it is unknown what level of increase should be considered problematic. Again, there is scarce data in the scientific medical literature to support thresholds on this subject.

Considering all of the above limitations, there is significant interest in the medical and scientific community to find biomarkers for renal tubular injury, given the perceived limitations of standard markers currently in use. Collection of these novel biomarkers in first-in-human and other phase 1 clinical trials could aid in the further development and research of appropriate biomarkers. More importantly, existing measures such as BUN and serum creatinine are also limited; notwithstanding the above limitations and the potential for false-negative results, the proposed composite biomarker has the potential to improve detection of clinical drug-induced kidney injury in a setting where prior evidence from experimental studies suggests that such a risk is present. Thus, while the submitter's data may help to provide further reassurance of the likelihood of the presence of renal tubular injury, when used, it should be used in conjunction with standard measures of renal function (sCr, BUN, urine albumin, and urine total protein) until additional more data can be obtained to further characterize the PFC index.

The submitter utilized the mesothelioma data set to validate the PFC index threshold established from the NHV study; however this is problematic given that the PFC index and COU is to be applied to other NHV populations and not those with cancer. While it is reassuring that this dataset was consistent with the PFC index derived from the NHV population, providing evidence that it can detect exposure to a nephrotoxicant, it does not help support the specific proposed COU.

5 RECOMMENDATIONS

It is the opinion of the Office of Clinical Pharmacology that the proposed PFC index can be qualified as a safety biomarker to be optionally used in conjunction with standard measures of renal function (sCr, BUN, urine albumin, and urine total protein) to inform decision-making in NHV phase 1 clinical trials with respect to detection of possible renal tubular injury.

Moreover, it is also the opinion of the Office that increases in any of the individual urine biomarkers in the PFC index beyond the range identified in the NHVs may also provide evidence of renal tubular injury in the context of nonclinical evidence of nephrotoxicity.

We recommend that the COU be revised to indicate that the PFC index of the 6 urine biomarkers, when used, is to be used in conjunction with standard measures of renal function (sCr, BUN, urine albumin, and urine total protein). The following additional limitations should also be reflected in the conditions of use: 1) the optimal sampling time to evaluate these biomarkers relative to the disposition of a nephrotoxicant has not been resolved and that introduces a potential risk for false negative findings; 2) the analytical performance characteristics of the assay used to develop the PFC index because the normal range was defined by a particular assay and PFC index may perform differently when used with assays that have different performance characteristics.