User's Guide[1]

Kidney Safety Composite Measure Biomarker for Use in Clinical Development[2]

Biomarker: Biomarker panel interpreted via a Composite Measure (CM) of the following six urinary biomarkers each normalized to urine creatinine (uCr): 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)

Context of Use: A safety composite biomarker panel to be used in conjunction with traditional measures to aid in the detection of kidney tubular injury in phase 1 trials in healthy volunteers when there is an a priori concern that a drug may cause renal tubular injury in humans.

[1]This User's Guide is authored by 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).

[2]Qualification granted by the U.S. Food and Drug Administration (FDA) on July 25, 2018 based on the Full Qualification Package for biomarker qualification DDTBMQ 000014 submitted under section 507 of the Federal Food, Drug, and Cosmetic Act by FNIH BC/PSTC NWG.

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1   Executive Summary

The Foundation for the National Institutes of Health Biomarkers Consortium (FNIH/BC) Kidney Safety Project Team and the Critical Path Institute's (C-Path) Predictive Safety Testing Consortium Nephrotoxicity Working Group (FNIH/BC/PSTC NWG) jointly submitted a full qualification package for a composite safety biomarker of renal tubular injury response to be used in normal healthy volunteer (NHV) trials during early drug development. The biomarker, herein referred to as the Composite Measure (CM), was qualified on July 25, 2018.

A CM of six (6) normalized urinary biomarkers, specifically 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), each normalized to urine creatinine (uCr), is a qualified safety biomarker of kidney tubular injury response for use in NHV trials supporting early drug development. This biomarker qualification represents the current thinking of the FDA on this topic (U.S. FDA 2018) and the sponsor's use of the CM as a drug development tool in well-controlled clinical studies is encouraged. In the context of a nonclinical nephrotoxicity signal, group-level increases of the CM above a threshold determined by an individual sponsor may indicate the potential presence of renal tubular injury.

> CLU is a secreted 76-80 kDa protein constitutively expressed at high levels during early stages of renal development and later in response to kidney injury in the proximal and distal tubules, glomerulus, and collecting duct (Rosenberg 1995). Expression of CLU mRNA is induced by different types of kidney injury in glomeruli, tubules and papilla of rats and dogs as a result of drug nephrotoxicity (Wadey 2014, Zhou 2014, Kharasch 2006, Rached 2008, Correa-Rotter 1998), surgery and ischemia (Nguan 2014, Tsuchiya 2005, Yoshida 2002, Ishii 2007), and in animal models of different renal diseases (Hidaka 2002).

> CysC is a protein marker that is freely filtered at the glomerulus and then reabsorbed by the renal tubular epithelium. In addition to its potential role in serum as a biomarker of glomerular filtration, CysC can also be measured in the urine in the presence of tubular dysfunction. An impairment of re-absorption in proximal tubules can lead to a several hundred-fold increase in urinary levels of CysC in humans and rats (Herget-Rosenthal 2007, Conti 2006).

> KIM-1 is a type I cell membrane glycoprotein. KIM-1 mRNA levels are elevated in kidney proximal tubule after initiation of kidney injury more than any other known gene across species (Ichimura 1998, Amin 2004). KIM-1 has proven to be one of the most promising biomarkers to monitor acute kidney injury (AKI) impacting proximal tubular epithelial cells due to rapidly increasing evidence of its pre-clinical and clinical utility in numerous contexts including its unique specificity, its sensitivity to detect various forms of tubular injury earlier than current diagnostic standards, its stability, and its translatability between different species.

> NAG is a 140 kDa lysosomal brush-border enzyme with two isoforms (A and B) mainly expressed in proximal tubules where its function is the breakdown of glycoproteins. Its excretion into urine correlates with increased tubular lysosomal activity and tubular cell injury (leakage). With AKI, and following treatment with nephrotoxic compounds, increased urinary NAG levels have typically been

observed before increases in serum creatinine (sCr) and blood urea nitrogen (BUN) (Sheira 2015, Westhuyzen 2003, Price 1992, Skálová 2005, Emeigh 2005, Ascione 1999). As a translational biomarker for drug development, NAG differs from the other drug-induced kidney injury (DIKI) biomarkers evaluated in this project, as it is not as consistently reliable in rodents.

NGAL, also known as human neutrophil lipocalin, lipocalin-2, siderocalin, or LCN2, is a 25-kDa protein initially identified in neutrophil specific granules. NGAL is expressed in various tissues at low levels and induced in epithelial cells with inflammation or other types of injury including malignancy (Cowland 1997). With kidney injury, NGAL is upregulated in the thick ascending limb of the loop of Henle, distal tubule and collecting duct, and is secreted into the urine as well as plasma (Paragas 2011). Because NGAL is rapidly upregulated following kidney tissue injury, it is a highly attractive biomarker for the sensitive monitoring of DIKI.

OPN, also known as secreted phosphoprotein I, sialoprotein I, or uropontin, is protective against oxidative stress and ischemia and has pro-inflammatory and profibrotic activity in the kidney (Fuchs 2011, Hudkins 1999). With tissue injury, OPN expression has been demonstrated throughout the kidney, and OPN has proven to be a very sensitive and inducible indicator of different forms of DIKI in animal studies (Lyle 2012).While investigations in renal transplant and critically ill patients support its utility for predicting patient outcome, the characterization of OPN in clinical kidney injury and disease settings is somewhat limited.

Use of the CM can help improve the development of safe and effective medicine where concern has been raised that an investigational drug may cause kidney injury. As stated in the FDA's Qualification Decision and Executive Summary, *"This biomarker can be used by drug developers for the qualified COU in submissions of investigational new drug applications (INDs), new drug applications (NDAs), and biologics license applications (BLAs) without the need to resubmit the biomarker information or rereview by the relevant [Center for Drug Evaluation and Research] CDER disciplines."* (U.S. FDA 2018)

1.1   Why is the Composite Measure important?

The kidney is a common target organ subject to drug toxicity. In nonclinical drug development safety assessment studies, kidney injury associated with drug toxicity can be defined by histopathology. Histopathology can be seen in such studies in the absence of changes in sCr or BUN. Often times, such changes may be seen only in one test species and, while human relevance may be questionable, a safe monitoring strategy to ensure clinical trial safety is needed before such compounds can advance to clinical development. Clinically, DIKI is detected by changes in standard clinical laboratory tests including sCr, BUN and serum cystatin C (sCysC). However, these traditional biomarkers are suboptimal when used for early signal detection of subtle injury in early clinical drug development settings. It is estimated that medically significant sCr elevation is not observed until >50% of renal tubule function is compromised. The CM has demonstrated added value for detecting DIKI at doses of known renal toxicants when no increase in sCr or BUN are observed, and at earlier time points than traditional biomarkers of renal tubular injury, which is critical to ensuring the safety of study subjects.

1.2   What is the Qualified Kidney Safety Composite Measure Biomarker?

The CM is a *measure of the geometric mean of the fold change from baseline* of urine CLU, CysC, KIM-1, NAG, NGAL, and OPN, each normalized to uCr. This CM has been qualified by the United States FDA for use in early clinical drug development as defined by the Context of Use (Section 3).

1.3   What should it be used for?

The group geometric mean (GM) CM of the CMs for all subjects in a specific dose cohort of an NHV study is qualified for sponsors to use to determine if there is an increased likelihood of a renal injury response to that dose of an investigational drug when benchmarked to results provided herein for NHVs. The individual subject CM is not currently qualified for individual subject safety monitoring.

The CM can be used for safety monitoring in clinical trials when nonclinical toxicology studies with a study drug demonstrate evidence of reversible histologic renal tubule damage that is associated with an elevation in any one or more of the six urine biomarkers.

The CM is qualified for use when standard biomarkers alone would be considered poor for initial detection of the renal tubule injury that has been observed in animal toxicology studies.

The CM is intended to complement the use of the standard biomarkers including sCr, BUN, urine albumin and urine total protein for safety monitoring in a single or multiple dose escalation clinical trial with or without a comparator/placebo during drug development under an Investigational New Drug Application (IND)/Clinical Trial Application (CTA).

1.4   Restrictions on use of the CM (i.e. not used for prospective NHV monitoring or for patient studies)

The CM is not intended to replace standard measures of renal function including current biomarkers and is not to be used for prospective real time NHV monitoring or for patient studies. The qualified CM is to be applied at the level of a study group or cohort and is not intended for individual subject safety monitoring.

1.5   How should it be used?

The observed group GM CM from subjects in a study drug dose group is used to assess if there is an increased likelihood of a renal injury response for that study drug at that dose and dosing regimen. The CM is calculated at each post-baseline timepoint. The full panel of six biomarkers and uCr must be measured at both baseline and the post-baseline timepoint for each subject. The urine specimen collection timepoints should be selected based on the onset of injury observed in the animal toxicology studies. At each post-baseline timepoint, the CM for each individual subject (referred to as timepoint-specific individual subject CM) is calculated as described in Section 4.1.1. The timepoint-specific group average CM (calculated as a Geometric Mean, GM See Section 4.1.2) for a drug study group alone, or relative to a comparator/placebo group, is then calculated to determine if the study drug group is consistent with what would be expected for NHVs.

1.6   How should the results be interpreted?

The application of the CM for renal injury response assessment is based on benchmarks of expected biomarker variability results observed for NHV subjects provided herein. Sponsors will need to define a probability level in a study protocol that would be considered unacceptable for the study drug relative to an NHV population. An appropriate probability level should be commensurate with the unmet medical need in the indication under investigation. The proposed probability level defines the threshold for the observed GM CM for a study drug dose, either alone or relative to a comparator/placebo group.

2   Background

Work to qualify nephrotoxicity biomarkers began in 2007 when the PSTC engaged the FDA, EMA and PMDA to discuss a large dataset of biomarker data generated using a number of nephrotoxic and non-nephrotoxic agents in rat studies benchmarked to histopathology. In 2008, FDA and EMA, and in 2010 PMDA, agreed that in preclinical studies urinary KIM-1, CLU, CysC, albumin, trefoil factor 3, total urinary protein, and β2-microglobulin were considered qualified for monitoring detection of acute DIKI with either tubular or glomerular involvement, and also agreed that the biomarkers could outperform and add value to sCr and BUN. The agencies also agreed with the recommendation that translational use of 5 of these 7 biomarkers in early clinical trial research may be appropriate on a case-by-case basis. Following this first qualification, the kidney biomarker work expanded to involve two parallel work streams: clinical studies in healthy volunteers and in patients exposed to standard of care agents known to be associated with renal injury, and more detailed examinations in rats, dogs and nonhuman primates including additional biomarkers.

The FNIH BC/PSTC NWG project team, selected six novel urine biomarkers, including CLU, CysC, KIM-1, NAG, NGAL, and OPN, for clinical qualification to be used with analytes such as urine albumin and urine total protein commonly used clinically to evaluate renal injury. Since the translational application of the individual biomarkers was not yet well characterized, utilizing all 6 safety biomarkers as a CM during clinical drug development was proposed as a more conservative option until further evidence of individual biomarker performance in the clinic is available. On July 25, 2018, the FDA Biomarker Qualification Program (BQP) qualified the CM to be used in conjunction with traditional measures to aid in the detection of kidney tubular injury in phase 1 normal healthy volunteer trials when there is an a priori concern that an investigational drug may cause renal tubular injury in humans. Further information about this qualification can be found at the [FDA BQP website](#).

3   Context of Use

Use Statement A safety composite biomarker panel to be used in conjunction with traditional measures to aid in the detection of kidney tubular injury in phase 1 trials in healthy volunteers when there is an a priori concern that a drug may cause renal tubular injury in humans (Figure 1).

Conditions of Qualified Use:

1. The CM is a measure of the fold change from baseline of urine CLU, CysC, KIM-1, NAG, NGAL, and OPN normalized to urine creatinine (uCr).

2. The group geometric mean (GM) CM is qualified for study sponsors to determine if there is an increased likelihood of a renal injury response for a dose of an investigational drug in a dose cohort when benchmarked to results provided herein for NHVs. The individual subject CM is not currently qualified for individual patient safety monitoring.

3. The CM is intended to complement the use of the standard biomarkers including sCr, BUN, urine albumin and urine total protein for safety monitoring in a single or multiple dose escalation clinical trial with or without a comparator/placebo during drug development under an Investigational New Drug Application (IND)/Clinical Trial Application (CTA).

4. The CM can be used for safety monitoring in clinical trials when nonclinical toxicology studies with a study drug demonstrate evidence of reversible histologic renal tubule damage that is associated with an elevation in any of the six urine biomarkers.

5. Urine for biomarker measurements should be collected at baseline and post-baseline to calculate the fold change from baseline. Sample collection times should be informed by animal toxicology study data for the study drug.

6. There should be a plasma drug exposure margin relative to the anticipated clinically relevant dose range, such that the likelihood of kidney injury is considered low at the doses proposed for clinical investigation. Alternatively, data that support greater understanding of potential species-specific mechanisms of questionable human relevance can contribute to confidence that likelihood of kidney injury is low in the proposed clinical investigation. As always, risk-benefit considerations are expected to contribute to exposure-margin based dose selection decisions.

7. The CM is qualified for use when standard biomarkers alone would be considered poor for initial detection of the renal tubule injury observed in animal toxicology studies. The CM is not intended to replace standard measures of renal function including current biomarkers as described in #3 above.

8. The CM is qualified for use in NHV studies.

9. The sponsor's use of the CM as a drug development tool in well-controlled clinical studies is encouraged.

4   Mode of Measurement

Drug development sponsors using the CM should collect urine at baseline and appropriate post-baseline timepoints to measure the full panel of six urine biomarkers (CLU, CysC, KIM-1, NAG, NGAL, and OPN) and uCr. uCr is measured in each of the same samples for biomarker normalization purposes.

Sponsors intending to use the CM in a clinical study submitted to regulatory authorities will be expected to provide performance characteristics of the biomarker research assays that meet or exceed the assays used in the qualification of this CM (see Appendix 1).

Figure 1: Proposed decision tree for clinical use of CM in Phase 1 NHV trials
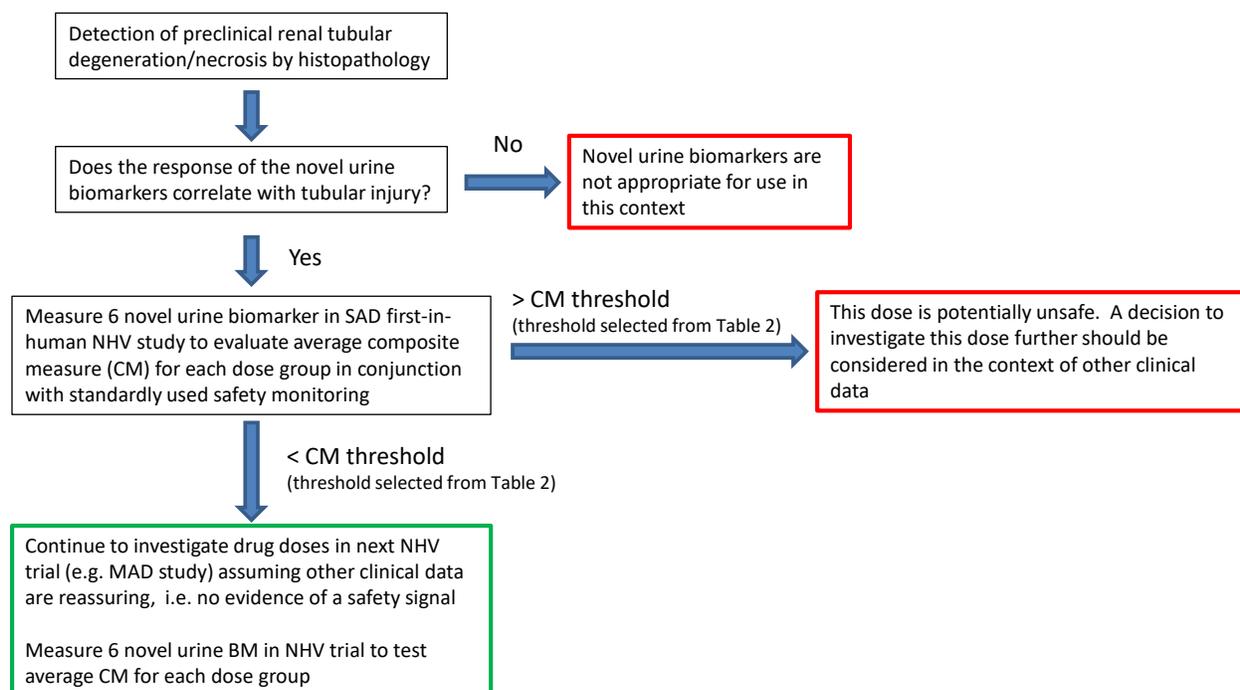
4.1   Methodology for sponsors to calculate and apply the CM for assessments of renal injury response in NHV trials

The observed group average CM from patients in a study drug dose group is used to assess if there is an increased likelihood of a renal injury response for that dose and dosing regimen of study drug. The CM is calculated at each post-baseline timepoint. The full panel of six biomarkers and uCr must be measured at both baseline and the post-baseline timepoint for each subject. At each post-baseline timepoint, the timepoint-specific individual subject CM will be calculated, as described in Section 4.1.1. The timepoint-specific group average CM (calculated as a Geometric Mean, GM See Section 4.1.2) for a drug study group alone, or relative to a comparator/placebo group, is then calculated to determine if the study drug group is consistent with what would be expected for NHVs.

The details for calculating the timepoint specific individual and group average CM are provided below, as well as instructions on how to apply the timepoint specific group average CM for safety monitoring when the study is designed to include, or not include, a comparator/placebo group (See Section 4.1.3). The application of the CM is based on the expected variability of the 6 biomarkers in a NHV population.

4.1.1   Instructions for calculating timepoint specific individual subject CM

Individual timepoint concentrations will be normalized to uCr for each biomarker and fold changes from baseline are calculated as follows:

- *CLU*: The normalized CLU concentration at a given timepoint is calculated as the concentration of CLU at that timepoint divided by the concentration of uCr at the same timepoint. The CLU fold change from baseline at a given timepoint is calculated as the normalized

CLU concentration at a given timepoint divided by the normalized CLU concentration at baseline.

- *CysC*: The normalized CysC concentration at a given timepoint is calculated as the concentration of CysC at that timepoint divided by the concentration of uCr at the same timepoint. The CysC fold change from baseline at a given timepoint is calculated as the normalized CysC concentration at a given timepoint divided by the normalized CysC concentration at baseline.

- *KIM-1*: The normalized KIM-1 concentration at a given timepoint is calculated as the concentration of KIM-1 at that timepoint divided by the concentration of uCr at the same timepoint. The KIM-1 fold change from baseline at a given timepoint is calculated as the normalized KIM-1 concentration at a given timepoint divided by the normalized KIM-1 concentration at baseline.

- *NAG*: The normalized NAG concentration at a given timepoint is calculated as the concentration of NAG at that timepoint divided by the concentration of uCr at the same timepoint. The NAG fold change from baseline at a given timepoint is calculated as the normalized NAG concentration at a given timepoint divided by the normalized NAG concentration at baseline.

- *NGAL*: The normalized NGAL concentration at a given timepoint is calculated as the concentration of NGAL at that timepoint divided by the concentration of uCr at the same timepoint. The NGAL fold change from baseline at a given timepoint is calculated as the normalized NGAL concentration at a given timepoint divided by the normalized NGAL concentration at baseline.

- OPN: The normalized OPN concentration at a given timepoint is calculated as the concentration of OPN at that timepoint divided by the concentration of uCr at the same timepoint. The OPN fold change from baseline at a given timepoint is calculated as the normalized OPN concentration at a given timepoint divided by the normalized OPN concentration at baseline.

The CM at a given timepoint, t, for an individual subject, i, is calculated as:

$$CM_{it} = exp\big[(1/6) \times \log(FC_{CLU,it}) + (1/6) \times \log(FC_{OPN,it}) + (1/6) \times \log(FC_{NAG,it}) \\ + (1/6) \times \log(FC_{KIM-1,it}) + (1/6) \times \log(FC_{CysC,it}) + (1/6) \times \log(FC_{NGAL,it})\big]$$

where $FC_{CLU,it}$, $FC_{OPN,it}$, $FC_{NAG,it}$, $FC_{KIM-1,it}$, $FC_{CysC,it}$, and $FC_{NGAL,it}$ are the CLU, OPN, NAG, KIM-1, CysC and NGAL fold changes from baseline for individual subject i, at timepoint t, as defined above. Note that log refers to the natural log transformation of the fold changes from baseline. In the event values are below the limit of quantitation, the value should be assigned as < LLOQ = LLOQ-0.1*LLOQ (i.e. 90% of the LLOQ value).

4.1.2 Instructions for calculating geometric mean (GM) CM of a drug study group or comparator/placebo group:

The GM CM for a drug study group or comparator/placebo group with n subjects at timepoint t is calculated as:

$$GM \ CM_t = exp\left(\sum_{i=1}^{n} \log(CM_{it})/n\right)$$

4.1.3   Instructions for applying the GM CM for safety monitoring:

Application of the CM is based on benchmarks of expected results from NHV subjects. **Sponsors will need to define a probability level in a study protocol that would be considered unacceptable for the study drug relative to an NHV population** (e.g., "at any post-baseline timepoint there is less than a 5% chance of observing such a result when the true underlying population is NHV"). Sponsors may propose a probability in a clinical protocol provided to the Agency. **<u>An appropriate probability level should be commensurate with the unmet medical need in the indication under investigation. The proposed probability level defines the threshold for the observed GM CM for a study drug dose, either alone or relative to a comparator/placebo group.</u>** Without a comparator/placebo group, an increased likelihood of renal injury response at a study drug dose may be declared if the observed GM CM of the drug study group is <u>inconsistent</u> with what would be expected in NHVs. With a comparator/placebo group, an increased likelihood of renal injury response at a study drug dose may be declared if the ratio of the observed GM CMs is <u>inconsistent</u> with what would be expected when comparing two NHV groups. An observed group GM CM for a study drug dose larger than the proposed threshold will be concluded to be associated with unacceptable probability for a potential renal injury response, and higher study drug doses may not be appropriate for further clinical investigation.

For example, Table 1 shows thresholds for the observed GM CM across various sample sizes (n/group) that would be expected with less than 5% probability from a drug study group consistent with a NHV population when assessing either the drug study group alone or relative to a comparator/placebo group. Please note that the thresholds in the tables are based on a single timepoint; they do not take into account use of the thresholds across multiple timepoints. This may represent a conservative analysis with a greater risk of declaring a false positive, i.e., incorrectly declaring a population consistent with NHV to be inconsistent with NHV, since there is no adjustment for multiplicity testing.

Table 1: Thresholds for the observed GM CM across various sample sizes (n/group) that would be expected with less than 5% probability from a drug study group consistent with an NHV population

Sample Size (n/group)	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than 5% probability in an NHV population	
	Threshold when assessing a drug study group alone GM CM	Threshold when assessing a drug study group relative to a comparator/placebo group (ratio of GM CMs)
6	1.28	1.33
8	1.25	1.27
10	1.23	1.24
12	1.22	1.22
14	1.21	1.20
16	1.20	1.19
18	1.19	1.18
20	1.18	1.16

GM = geometric mean
CM = composite measure of the fold change from baseline of urine CLU, CysC, KIM-1, NAG, NGAL and OPN, normalized to urine creatinine

A sponsor developing a study drug with a life-saving potential in patients with few treatment options may consider alternate probabilities that are associated with larger deviations from NHVs. The thresholds on the observed GM CM associated with the alternate probability levels are provided for NHVs when assessing either the drug study group alone Table 2 or relative to a comparator/placebo group (Table 3), for various sample sizes (n/group).

Table 2: Observed GM CM thresholds when assessing a drug study group *alone* with an NHV population (samples sizes of n = 6 to 20 per group)

Sample	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than P% probability in an NHV population				
Size (n/group)	P = 50%	P = 20%	P = 10%	P = 5%	P = 1%
6	1.07	1.18	1.23	1.28	1.38
8	1.07	1.16	1.21	1.25	1.33
10	1.07	1.15	1.19	1.23	1.30
12	1.07	1.14	1.18	1.22	1.28
14	1.07	1.14	1.18	1.21	1.26
16	1.07	1.13	1.17	1.20	1.25
18	1.07	1.13	1.16	1.19	1.24
20	1.07	1.13	1.16	1.18	1.23

GM = geometric mean

CM = composite measure of the fold change from baseline of urine KIM-1, CLU, OPN, CysC, NAG, and NGAL normalized to uCr

Table 3: Observed ratio of GM CM thresholds when assessing a drug study group *relative to a comparator/placebo* with an NHV population (samples sizes of n = 6 to 20 per group)

Sample	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than P% probability in an NHV population				
Size (n/group)	P = 50%	P = 20%	P = 10%	P = 5%	P = 1%
6	1.00	1.15	1.24	1.33	1.49
8	1.00	1.12	1.20	1.27	1.42
10	1.00	1.11	1.18	1.24	1.37
12	1.00	1.10	1.16	1.22	1.34
14	1.00	1.10	1.15	1.20	1.30
16	1.00	1.09	1.14	1.19	1.28
18	1.00	1.08	1.13	1.18	1.26
20	1.00	1.08	1.12	1.16	1.25

GM = geometric mean
CM = composite measure of the fold change from baseline of urine KIM-1, CLU, OPN, CysC, NAG, and NGAL normalized to uCr

5   Clinical Data Used to Support CM COU

Six novel urine biomarkers were selected for safety monitoring in clinical trials during drug development based on clinical study data. The six urine biomarkers include CLU, OPN, NAG, KIM-1, CysC, and NGAL, each normalized to uCr. Urine biomarker data from the PSTC NHV study were used to derive the CM thresholds. Data to support the derivation and qualification of the CM are derived from two clinical studies:

- NHV data from the PSTC NHV Study: Biomarker data were collected from 81 NHVs at two visits approximately 3 weeks apart. The data used to derive the CM and define normal variability CM thresholds were from 76 of the NHV who had data on all six biomarkers at both visits.

- Data from cisplatin treated patients in a Mesothelioma Study: Biomarker data were collected from 39 patients without evidence of chronic kidney disease (CKD) at baseline with a median of 8 (range 4-9) post-baseline visits within 6 days of surgery. Three subgroups were defined in the Mesothelioma Study, including Meso Surgery (N = 4 surgical control patients without

exposure to cisplatin), Meso Controls (N = 22 patients exposed to cisplatin without clinical manifestation of treatment related renal injury [i.e., patients could have maximum increases in sCr <50% and <0.3 mg/dL above baseline]), and Meso Cases (N = 13 patients exposed to cisplatin with clinical manifestation of treatment related renal injury [i.e., increases in sCr >50% and/or >0.3 mg/dL above baseline]). The CM calculated for the mesothelioma subgroups were used to assess the performance of the CM in patients with known exposure to the nephrotoxicant cisplatin and acute kidney injury. The results from this study can be found at the FDA BQP website.

6   Appendix 1:   Biomarker Assay Characteristics

The assays used to support this qualification were developed in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory and validated as Laboratory Developed Tests (LDT). The protocols underwent a fit-for-purpose validation for their proposed COU. All urine biomarker data that supported the qualification was generated at Pacific Biomarkers Incorporated (PBI). Additional information about the assays is provided below and in Table 4.

6.1   Clusterin (CLU)

The CLU assay is a commercially-available immunoassay from R&D Systems using manufacturer provided recombinant human CLU as the calibrator and in-house controls prepared from urine from single donors. The average within-run precision CV was <11% and the average between-run precision CV was <11% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted up to 4-fold in addition to the standard 1:4 initial assay dilution. The assay had an overall percent recovery of 100.9% (range 90.0-107.5%) using recombinant CLU. The lower limit of quantitation (LLOQ) (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 2.5 ng/mL (corresponding to a reportable level of 10 ng/mL correcting for the 1:4 standard assay dilution). The reference interval for PBI samples normalized to uCr was determined to be 35-383 ng CLU/mg uCr.

6.2   Urinary Cystatin C (CysC)

The CysC assay is a commercially-available immunoassay from R&D Systems using manufacturer provided recombinant human CysC as the calibrator and in-house controls prepared from urine from single donors. The average within-run precision CV was <5% and the average between-run precision CV was <12% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted up to 64-fold. The assay had an overall percent recovery of 96.5% (range 83.8-104.2%) using recombinant CysC. The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 1.4 ng/mL. The reference interval for PBI samples normalized to uCr was determined to be 0.014-0.058 µg CysC/mg uCr. In limited stability evaluations, samples were stable through one freeze/thaw.

6.3   Kidney Injury Molecule-1 (KIM-1)

The KIM-1 assay is a commercially-available immunoassay from R&D Systems using manufacturer provided recombinant human KIM-1 as the calibrator and in-house controls prepared from urine from single donors. The average within-run precision CV was <9% and the average between-run precision CV was <16% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted up to 32-fold. The assay had an overall percent recovery of 107.0% (range 96.6-118.0%) using recombinant KIM-1. The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 11.6 pg/mL. The reference interval for PBI samples normalized to uCr was determined to be 0.117-1.351 ng KIM-1/mg uCr. In limited stability evaluations, samples were stable through three freeze/thaws.

6.4   N-Acetyl-beta-D-Glucosaminidase (NAG)

The NAG assay is a commercially-available colorimetric assay from Roche Diagnostics using manufacturer provided NAG standards as the calibrator and in-house controls prepared from pooled human urine, human urine spiked with NAG standard and bovine NAG in deionized water. The average within-run precision CV was <5% and the average between-run precision CV was <6% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted 1:3. The assay had an overall percent recovery of 102.1% (range 99.1-104.5%) using the NAG standard. The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 0.31 U/L. The reference interval for PBI samples normalized to uCr was determined to be <0.78 U NAG/mg uCr. In limited stability evaluations, samples were stable through three freeze/thaws.

6.5   Neutrophil Gelatinase-Associated Lipocalin (NGAL)

The NGAL assay is a commercially-available immunoassay from BioPorto Diagnostics using manufacturer provided NGAL standards as the calibrator and in-house controls prepared from urine from single donors. This assay is approved for *in vitro* diagnostic use in the European Union, Brazil, Canada, Chile, Iran and India. The average within-run precision CV was <10% and the average between-run precision CV was <7% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted up to 64-fold in addition to the standard 100-fold initial assay dilution. The assay had an overall percent recovery of 103.1% (range 93.3-109.4%) using the NGAL standard. The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 0.4 ng/mL (corresponding to a reportable level of 40 ng/mL correcting for the 100-fold standard assay dilution). The reference interval for PBI samples normalized to uCr was determined to be <41.8 ng NGAL/mg uCr. In limited stability evaluations, samples were stable through four freeze/thaws.

6.6   Osteopontin (OPN)

The OPN assay is a commercially available immunoassay from R&D Systems using manufacturer provided controls and recombinant human OPN as the calibrator and in-house controls prepared from pooled human urine. The average within-run precision CV was <4% and the average between-run precision CV was <13% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted up to 32-fold in addition to the standard 400-fold initial assay dilution. The assay had an overall percent recovery of 99.9% (range 97.9 -101.5%) using recombinant OPN. The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 0.10 ng/mL (corresponding to a reportable level of 40 ng/mL correcting for the 400-fold standard assay dilution). The reference interval for PBI samples normalized to uCr was determined to be 495-2,029 µg OPN/mg uCr. In limited stability evaluations, samples were stable through four freeze/thaws.

6.7   Urinary Creatinine (uCr)

The uCr assay is a commercially-available enzymatic assay approved by the FDA for *in vitro* diagnostic use. This assay is standardized against ID-MS and the primary reference material used for the assay is the U.S. NIST SRM 914. The assay uses manufacturer provided calibrator and controls and an in-house control prepared from a serum pool. The average within-run precision CV was <1% and the average between-run precision CV was <3% using controls. The assay was shown to be linear without degradation of response so that samples with a high concentration were able to be diluted 5-fold. A urine control with an elevated concentration of uCr was used in recovery evaluations and the assay had an overall percent recovery of 105.1% (range 103.5-107.9%). The LLOQ (estimated based on the lowest concentration that can be measured with an inter-assay CV of 20%) was determined to be 0.8 mg/dL. The reference interval for PBI samples was determined to be 40.0-278.0 mg/dL for males and 29.0-226.0 mg/dL for females. In limited stability evaluations, samples were stable through four freeze/thaws.

Table 4: Urine Biomarker Assay Method and Manufacturer used by PBI

Assay	OPN	KIM-1	CysC	Clusterin	NGAL	NAG	Total Protein	Albumin	Cr
Method	ELISA	ELISA	ELISA	ELISA	ELISA	Colori-metric	Turbidi-metric	Immuno-turbidimetric	Enzymatic
Manu-facturer	R&D Systems	R&D Systems	R&D Systems	R&D Systems	BioPorto Diagnostics	Roche Diagnostics	Roche Diagnostics	Roche Diagnostics	Roche Diagnostics

7   Appendix 2: References

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8   Appendix 3: Revision History

Revision History		
Date	**Version**	**Summary of Revisions**
January 2019	1.0	Initial version
May 15, 2019	1.1	Section 4.1.1: Applying CM with LLOQ values