DRUG DEVELOPMENT TOOL
QUALIFICATION PLAN
DETERMINATION LETTER
DDT-BMQ-000075

December 6, 2024

Critical-Path Institute
Attention: Nicholas King
1730 E. River
Tucson, AZ 85718

Dear Mr. King:

FDA has completed its review of the Qualification Plan (QP) for Drug Development Tool (DDT), **DDT-BMQ-000075-QP-2**, received on May 8, 2024, by the CDER/CBER Biomarker Qualification Program (BQP), submitted under section 507 of the Federal Food, Drug, and Cosmetic (FD&C) Act.[1]

The QP is for a safety urine biomarker panel to be used in conjunction with standard renal safety laboratory tests (e.g., sCr, BUN, and sCysC) to indicate response to drug-induced injury to the renal tubule in individuals with normal renal function enrolled in early phase drug development clinical trials.

FDA has completed its review and has agreed to **accept** your QP. Note that projects in the Biomarker Qualification Program may be eligible to apply for DDT research grant programs, which are offered for further DDT development as funding permits.[2]

In preparing to submit a Full Qualification Package (FQP), ensure that the FQP submission addresses the scientific issues and the recommendations outlined in the Appendix section of this letter.

[1] In December 2016, the 21st Century Cures Act added section 507 to the FD&C Act. FDA is now operating its DDT programs under section 507 of the FD&C Act.
[2] RFA-FD-24-030: Drug Development Tools Research Grants (U01) Clinical Trial Optional.

Should you have any questions, contact the CDER Biomarker Qualification Program at CDER-BiomarkerQualificationProgram@fda.hhs.gov and refer to **DDT-BMQ-000075** in the subject line of your email.

Sincerely,

Vanitha J. Sekar -S

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Date: 2024.12.06 06:32:10 -08'00'

Vanitha Sekar, PhD
Director, Division of Biomedical Informatics,
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Aliza M. Thompson -S

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APPENDIX

RECOMMENDATIONS AND CONSIDERATIONS

Context of Use (COU) Considerations

1. As part of the COU, you have provided a definition for normal renal function. The term “normal renal function” can be interpreted in different ways. In place of “normal renal function,” FDA recommends referring to individuals, *“with an estimated or measured glomerular filtration rate of ≥60 mL/min/1.73m2 and urinary albumin creatinine ratio that is <30mg/g.”*

2. To reflect the primary analysis method of evaluation of this biomarker qualification and intended use, the FDA recommends that you add *“in conjunction with clinical judgement and standard renal safety laboratory tests”* into the COU.

   Recommended COU: The safety biomarker panel is to be used in conjunction with the results of standard renal safety laboratory tests and clinical judgment to identify drug-induced injury to the renal tubule in adults with an estimated or measured glomerular filtration rate of ≥60 mL/min/1.73m2 and urinary albumin creatinine ratio that is <30 mg/g enrolled in early phase drug development clinical trials. The use of the biomarkers (specific biomarker selection, timing, exposure to safety margins, etc.) will be guided by the results of the nonclinical studies conducted using the same drug.

Analytical Considerations:

We strongly encourage you to provide detailed responses and/or data to address the analytical issues noted below, as this information will be critical for our review of your FQP.

3. In response to question 7 in the information request, you state that, in the future, the laboratory will be responsible for the validation of the assay, including the precision and upper limit of quantification (ULOQ). You also state that precision testing was conducted on native samples from healthy volunteers, and it was not possible to use the intended disease population for these studies. While using native samples is ideal, the performance characteristics of your assay should be well characterized for the range of values that will be encountered in this qualification effort. In your FQP, please state if the performance testing covers the ranges of values for the samples in this qualification effort. If not, provide data that evaluates the performance of these assays for the expected biomarker values of all samples in this qualification effort. Ensure that you have tested the measurement range of the assays that will be used to evaluate the samples from the clinical study in your qualification project. These performance characteristics need to be evaluated for the full range of expected values to ensure your assays will support your statistically significant and medically significant thresholds.

4. In the Letter of Intent (LOI) determination letter, you were asked to explain how the analytical validation of the novel urinary biomarkers (CLU, Cys C, KIM-1, NAG, NGAL, and OPN), excluding urinary albumin and protein, is sufficient for decision making on an individual study subject basis. In the previous qualification, you submitted validation data to support a COU for a cohort of healthy volunteers which can be helpful for the COU in this project, but additional explanation and data are needed. In your response to the reviewability memorandum (received December 12, 2023) you stated that, "Analytical validation of the six novel biomarker assays was carried out in a CLIA-licensed laboratory," and that the results from these research-use-only assays "compared favorably with cleared 510(k) immunoassays for clinical laboratory utilization and patient decision making." However, it is unclear from your response how the analytical validation of your assays (summarized in Tables 8 and 9 of your QP) is sufficient for decision making, especially in the context of the proposed urine creatinine- normalized statistically and medically significant thresholds for use that were summarized in Table 12 of your QP. In your FQP, provide more detail describing how the analytical validation of the novel urinary biomarkers (CLU, Cys C, KIM-1, NAG, NGAL, and OPN) is sufficient for decision making on an individual study subject basis and how it supports your COU taking into consideration sources of error in analytical performance for each biomarker, variability due to urine creatinine measurement and normalization, and proposed creatinine normalized statistically significant and medically significant thresholds.

5. In the information request sent on April 24, 2024, you were asked to describe the risk analysis you performed to determine that cisplatin and tobramycin were the only exogenous substances necessary to study for interference and why the risks of inaccurate results were considered low for other common exogenous compounds. You were also asked if you had considered any other comorbid conditions that could impact the accuracy of your tests because they affect the biomarkers you are measuring and could lead to incorrect results. In your response, received on May 8, 2024, you stated that cisplatin and tobramycin were the only exogenous substances tested for interference and indicated that it was "unclear if a risk analysis was performed".

   Based on your response, it is unclear if there are other exogenous substances which could interfere with your assays and impact the clinical results. For example, your mesothelioma cisplatin study (which, along with your Normal Healthy Volunteer (NHV) study, was used to generate clinical data to establish statistically significant and medically significant thresholds for each novel biomarker) used the drug cisplatin. However, it is unclear if the subjects in your mesothelioma cisplatin study were being treated with other drugs that were not assessed for interference. Similarly, it is unclear if the subjects in your aminoglycoside confirmatory phase study were being treated with any other exogenous substances which could have had an impact on your assays' results.

It is also unclear if a risk analysis was conducted and if other common exogenous substances that may have the potential to interfere were tested. There is uncertainty about the performance of your assays, as well as the statistical and medical thresholds you determined, because some subjects who were used to determine these thresholds may have been exposed to exogenous drugs or may have had comorbid conditions which may have interfered with your assays.

In your FQP, provide a detailed risk analysis assessing exogenous compounds and comorbid conditions for potential assay interference. If you determine that a substance or comorbidity does not have the potential to interfere with your assay, describe the scientific basis for how you made that determination. For substances and comorbidities which you determine could potentially interfere with your assay, describe how you will account for this interference in your threshold determination and analysis of samples.

6. In the information request submitted on April 24, 2024, FDA asked you to describe any mitigations you intend to implement to address the risk of inaccurate (false negative or false positive) results due to interference (for example, from hemolysis). FDA also asked you to address why any unmitigated interference will not introduce unacceptable error and result in inaccurate results. In your information request response received on May 8, 2024, you discussed visual inspection as mitigation for hemolysis leading to inaccurate results. However, in this response you did not provide evidence of implementation of this mitigation or evidence that users would be able to tell if a sample is hemolyzed. FDA is concerned that samples could be hemolyzed without being detectable by visual inspection. In your FQP, provide additional information to demonstrate the effectiveness of the risk mitigations and data to support that users would be able to visually determine if a sample has been hemolyzed.

7. In Table 8 in the QP, you indicate that you used two 510(k) cleared Roche Modular P assays and one Roche c501 assay for creatinine. In your interactive review response received on May 8, 2024, you were unable to obtain the 510(k) numbers in time to response. In your FQP, provide the 510(k) numbers (or the trade names and/or the labeling) for the three creatinine 510(k) cleared assays used in your evaluation. If you are unable to provide the 510(k) numbers for these assays, confirm that all creatinine testing was conducted using 510(k) cleared assays.

8. In the analytical data submitted in your QP in support of your assays, there are multiple potential sources of analytical error such as interference from various substances, imprecision, variability across long-term storage and stability conditions, etc. However, it is unclear from your QP how multiple sources of potential analytical error observed in your analytical performance studies for both your RUO and 510k cleared assays may impact the total analytical error budget in the context of the creatinine-normalized fold changes relative to the statistically significant and medically significant thresholds you have established. For example, some source of analytical error for the KIM-1 RUO assay in Table 9 of your QP include, for the low

and high KIM-1 biomarkers respectively, 64% and 27% interference for Albumin (HSA) and 112.9% and 46.2% interference for Packed Erythrocytes at 1%. The between run precision CV's ranged from 11.3% for the low sample to 15.8% for the high sample. The long-term stability at -80°C for KIM-1 was 44 months based on the acceptance criteria of 30% difference from baseline for KIM-1. Stability at a time point was met if >67% of the samples met the stability acceptance criteria, and an individual sample was considered unstable if it did not meet the acceptance criteria for two consecutive time points. However, within that 44-month stability time frame, the percent difference from baseline varied by as much as +40% from baseline for samples at some time points to -60% from baseline for samples at other timepoints.

It is unclear from your QP what impact these combined different sources of variation (i.e., the total analytical error) in your analytical studies for your RUO and 510k cleared assays may have on your proposed statistically significant and medically significant thresholds considering that these different sources of error may be additive. This increased total analytical error, if positive, could cause a single biomarker (like KIM-1, for example) or a combination of multiple biomarkers to cross the statistically or medically significant thresholds and lead to a false positive result. Conversely, if the total analytical error is additive in the negative direction, this could lead to a false negative result (i.e., one or more biomarkers erroneously do not cross either the statistically or medically significant threshold due to this negative total error). In your FQP, for each assay/biomarker, provide an analysis (such as modeling, for example) of the different sources of error (such as, but not limited to, interference, variations in long term stability, imprecision, etc.) in the context of the total acceptable error budget and what impact this total error has (or does not have) on the creatinine normalized values both in terms of your statistically and medically significant fold change thresholds and your COU.

9. You included the following text in the "Long-Term Stability Assessment Statement for the Translational Kidney Tubule Safety Biomarker Panel (Extended) Context of Use" document: "our confirmatory study samples were not analyzed until samples were stored at -70°C or colder for up to 46 months for CLU, and 45 months for OPN." However, according to your Long-Term stability study, CLU is only stable for 3 months and OPN is only stable for 12 months. In your FQP, provide evidence that justifies the use of samples that were beyond the stability limits for your RUO assays for your confirmatory study.

Clinical/Statistical Considerations

10. You state that no formal approach will be used to address any missing values. Address the following in your submission:

    - Explain how missing data will affect the interpretability of the primary and secondary analysis results.

    - You state in the SAP that "All available data will be used to derive the exposure endpoints as described in Section 2.3.4. If the patient is deemed

part of the PP population (reference Section 2.4.1) for a given Primary or Secondary exposure endpoint, then there is sufficient data to make an efficient evaluation of potential exposure to a nephrotoxicant for an individual patient. Further details on the data requirements for inclusion in the PP population are provided in Appendix 1." Appendix 1 was not submitted to the Agency. Provide Appendix 1 and explain what defines sufficient data to conduct an efficient evaluation.

- Provide descriptive statistics to summarize missing data in subjects who are included in the per-protocol population and who are excluded from the per-protocol population, respectively.

11. In the QP, you state that this project will demonstrate that these biomarkers reliably change in humans, just as seen in animals, in response to an acute drug-induced tubular renal injury prior to irreversible kidney injury at lower drug exposures or earlier time points than sCr, BUN, and sCysC. It is unclear how the data from the confirmatory phase studies will be used to demonstrate these biomarkers detect acute tubular injury at earlier time points or at lower drug exposure. Please address in your FQP and include in the SAP for FQP review if statistical approaches are planned to address this issue.

12. For the learning phase mesothelin-cisplatin study, provide detailed information for groups 2 and 3 separately. Provide information as has been shown in Table 27 row "Meso Controls (minus Gr4)" on the biomarkers that increased in groups 2 and 3 for the statistically significant threshold and medically significant threshold.

13. Provide the ROC curves and analysis for calculating the statistical significant and medically significant thresholds of fold change (FC) from baseline for each novel biomarker. In addition, for Table 24, provide the details on how sensitivity and specificity were obtained for each biomarker, and the derivation of the upper limit FC from baseline and the choice of threshold of FC for statistically significant thresholds. Similarly, for Table 25, provide the details on how sensitivity and specificity were obtained for each biomarker and the choice of threshold of FC for medically significant thresholds.

14. For the primary analysis, provide your rationale for the choice of 80% as the lower bound of the 95% confidence interval for the specificity to identify subjects not treated with a known nephrotoxic drug.

15. For the secondary analyses, explain how you have determined the subsets of 6 biomarkers, 4 biomarkers and 3 biomarkers that would be included. For example, provide the clinical rationale for excluding CysC and total protein from the subset of 6 biomarkers as opposed to two other biomarkers from the 8 biomarkers when there are multiple possibilities for the exclusion of two biomarkers.