LOI DETERMINATION LETTER

DDTBMQ000081 – Amendment 1

May 13, 2020

Critical Path Institute – Predictive Safety Testing Consortium and Duchenne Regulatory Science Consortium
1730 E. River Rd., Suite 200
Tucson, AZ 85718

Dear Dr. Sauer:

We are issuing this Letter of Intent (LOI) Determination Letter to the Critical Path Institute's Predictive Safety Testing Consortium (PSTC) Skeletal Muscle Working Group and Duchenne Regulatory Science Consortium (D-RSC), regarding your proposed qualification project submitted to the Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program (BQP). We have completed our review of your LOI submission that was accepted for review on December 20, 2019, and have concluded to **Accept** it into the CDER BQP[1]. We support and encourage your ongoing study and expanding the use of this promising safety biomarker panel to aid in the detection of acute drug induced skeletal muscle injury in phase 1 trials.

You have proposed qualification of a panel of four biomarkers in the plasma [Skeletal troponin I fast-twitch Type II (TNNI2), Myosin light chain 3 (MYL3), Fatty-acid binding protein 3 (FABP3), Creatine kinase muscle type (CKM)] as safety biomarkers to aid in the detection of acute drug induced skeletal muscle injury in phase 1 trials in healthy volunteers in conjunction with aspartate transaminase (AST) and total creatine kinase (CK) enzymatic activity when there is an a priori concern that a drug may cause skeletal muscle injury in humans. As this biomarker development effort is refined in subsequent submissions, the submitted data, the specifics of your context of use (including the target patient population), and the design of study(ies) used in the clinical validation of the biomarker will ultimately determine which of the recommendations below are most applicable.

Based on our review of the LOI, we agree there is an unmet need and agree that development of the proposed panel of safety biomarkers would potentially enable detection of drug induced skeletal muscle injury before elevation of current standards (AST and CK) mentioned above.

For the 507 DDT qualification process, please prepare a Qualification Plan (QP) submission that addresses the scientific issues and the recommendations outlined below. A QP contains details

[1] In December, 2016, the 21st Century Cures Act added section 507 to the Food, Drug, Cosmetic Act (FD&C Act). FDA is now operating its drug development tools (DDT) programs under section 507 of the FD&C Act.

of the analytical validation of the biomarker measurement method, detailed summaries of existing data that will support the biomarker and its context of use (COU), and descriptions of knowledge gaps and how you propose they will be mitigated. If future studies are planned, please include detailed study protocols and the statistical analysis plan for each study as part of your QP submission.

When evaluating biomarkers prospectively in clinical trials, sponsors are encouraged to submit study data using Clinical Data Interchange Consortium (CDISC) standards to facilitate review and utilization of data. Data sharing and the capability to integrate data across trials can enhance biomarker development and utilization. If sponsors intend to include analyses of these biomarkers to support regulatory decision making for a specific Investigational New Drug (IND) development program, they should prospectively discuss the approach with the appropriate CDER division. Any groups (academia, industry, government) that would like to join in this effort or have information or data that may be useful can contact Dr. John-Michael Sauer, PhD (jsauer@c-path.org), the point of contact for this project, or view the Critical Path Institute website.

Biomarker Considerations

Requestor's Description: *A panel of 4 biomarkers including:*

Acronym	Name (Unique ID (Uniprot))
TNNI2	Skeletal troponin I fast-twitch (Type II) - (P48788)
MYL3	Myosin light chain 3 – (P08590)
FABP3	Fatty-acid binding protein 3 – (P05413)
CKM	Creatine kinase muscle type – (P06732)

FDA's questions for continued development of the biomarker description:
We agree with your description of the above biomarkers. However, the method of interpretation of these biomarker panel is still not clear. When submitting your QP, please provide more clarity on how the biomarker panel will be assessed and interpreted. Please include a rationale for any thresholds, cut-offs, and decision algorithms proposed.

Context of Use (COU) Considerations

Requestor's COU: *A safety biomarker panel to aid in the detection of acute drug induced skeletal muscle injury in phase 1 trials in healthy volunteers in conjunction with aspartate transaminase (AST) and total creatine kinase (CK) enzymatic activity when there is an a priori concern that a drug may cause skeletal muscle injury in humans.*

FDA's suggested COU for continued biomarker development: We agree with your suggested COU. In your QP submission, please provide specifics regarding what is an *a priori* concern and how it will trigger the novel biomarker panel assessment.

In preparing to submit a Qualification Plan (QP), please ensure that the QP submission addresses the scientific issues and the recommendations outlined below.

Analytical Considerations

1. In your LOI, you indicated that the biomarkers are measured using Research Use Only sandwich ELISAs from Meso Scale Diagnostics. You indicated that a fit for purpose validation of the assays has been conducted for human samples, evaluating intra-and inter-assay precision, dilutional linearity, limit of blank (analytical sensitivity), limits of quantitation (upper and lower), matrix recovery, and sample-free/thaw stability. We did not identify a description or summary data of validation protocols which utilized human samples, so we are unable to comment on their adequacy to analytically validate the use of these ELISAs for measurement of your biomarkers in human samples. However, we note that you previously provided a description of analytical validation for assays to measure these biomarkers in rat serum as part of the SKM Briefing Book "Qualification of Skeletal Muscle Injury Biomarkers in Rat", and have the following comments for your validation of biomarker assays for measurement of human samples (or measurement of samples from any additional animal model you intend to claim) based on the analytical studies described in that document.

    a. To determine the Limit of Detection and Limit of Quantification, you evaluated samples which appear to be the same as those which you have used to calibrate your device (device standards). The use of these standards to determine the Limit of Detection and Limit of Quantification, which were also used to calibrate the device, would over estimate device performance (and in practice, would not represent the limit of detection and quantification associated with samples users would measure). If samples of low concentration (near the Limit of Detection and Limit of Quantitation) are important for the context of use, clinical samples should be used when determining the limit of detection and quantification of your assays.
    b. Your acceptance criteria for these studies appear broad. We recommend that you consider what performance is needed to support the context of use of these biomarkers and determine acceptance criteria for validation studies based on the performance you determine necessary for your specific context of use. We recommend that you define acceptance criteria for each analytical validation study in the context of the cumulative effect that different sources of error, including bias or systematic differences as well as imprecision, have on device performance. You should define acceptance criteria such that your total analytical error does not preclude the determination of clinically meaningful differences in the biomarkers so that you can reliably differentiate between samples with biomarker concentrations that indicate drug induced skeletal muscle

degeneration/necrosis, and samples with biomarker concentrations that indicate no drug induced skeletal muscle degeneration/necrosis.

c. Your sample stability studies should demonstrate that no conditions which samples are subject to, both in your analytical studies, as well as in the anticipated use of the biomarker, introduce changes that could negatively impact the interpretation of results. For example, in both your frozen sample stability studies as well as your freeze thaw sample stability studies, it appears that you have not compared results obtained in these studies to fresh samples (and instead compare results to those measured from samples after an initial thaw). If you intend to measure fresh (unfrozen) samples as well as samples which have been frozen and thawed within your context of use, you should analytically confirm that measurements of fresh (unfrozen) samples are comparable to samples which have been frozen and thawed.

Clinical Considerations

1. Based on the limitations of AST and CK in determining SKM toxicity and the nonclinical data already collected, we recommend that prior to pursuing a qualification in the clinical setting, where you will have to rely on AST and CK to validate your biomarkers, you may consider pursuing the qualification of your biomarker panel in the non-clinical setting. In this setting, you will be able to use the histopathology data to confirm your hypothesis that these biomarkers increase in response to drug-induced SKM toxicants, correlate with the severity of muscle injury, and return to baseline on recovery from SKM injury. In addition, you will be able to explore important clinical questions, such as whether this set of biomarkers can specifically identify drug-induced skeletal muscle injury and differentiate those events from other causes of skeletal muscle injury or muscle enzyme elevation, such as exercise. If you choose to qualify your biomarker panel in a non-clinical setting, you would need to revise your COU accordingly or submit a separate LOI.

2. The use of the proposed plasma/serum biomarkers to detect SKM degeneration / necrosis was primarily evaluated in rats. Before you continue to clinical studies, we would prefer evaluating the use of these biomarkers to detect drug-induced SKM degeneration / necrosis in other nonclinical species commonly used to evaluate drug toxicity, including mice, dogs, and macaques.

3. As stated above, histopathology data in animals will be necessary to correlate the biomarker to injury. The validity of the biomarker panel will be increased if qualified in pre-clinical studies first using histopathology data to link the muscle injury to serum changes in the biomarkers. We strongly recommend that you first pursue the qualification of your biomarker panel in the non-clinical setting. If you decide to continue with the clinical qualification, there will need to be substantial non-clinical information submitted for review to support the clinical relevancy of the proposed COU.

4. You comment that SKM injury is currently monitored in clinical drug development trials and in muscular and neuromuscular diseases using CK and AST, and that these biomarkers

lack the desired sensitivity and tissue specificity. However, you envision your panel of biomarkers as a supplement to AST and CK measurements and are, in part, relying on these imprecise AST and CK measurements to validate your new panel of biomarkers. How do you plan to address this issue of validating biomarkers by comparing to imperfect standards?

5. You state that it is "assumed that the four biomarkers will translate from preclinical to clinical, based on the clinical data already generated and the fact that nonclinical performance is correlated with histopathology rather than mechanism of toxicity or pathogenesis of disease". You also state that "nonclinical data correlating the biomarker response to SKM histopathology has been generated". The wording of these statements are a little bit confusing. Please confirm if you wish to state that the clinical data that will be generated will be supported by the fact that non-clinical data correlate with the SKM histopathology.

6. In considering what clinical data would be needed to support the proposed SKM biomarkers, we anticipate that you will need to demonstrate the performance of the biomarkers by collecting samples prospectively in one or more drug development programs where subjects experience drug-related myopathy/rhabdomyolysis and, at least some, recover from that injury.

7. It will be important to know if and when these biomarkers return to baseline once the patient has recovered from drug-induced SKM injury. It would be helpful to know if the biomarker panel has the ability to predict clinically meaningful skeletal muscle injury, instead of simply to detect previous injury, and if the panel can be used to determine degree or severity of injury. It will also be essential to demonstrate that this biomarker panel is not elevated when injury has not occurred, or injury has occurred but resolved.

8. In your QP, incorporate plans to address the following questions related to clinical utility of your biomarker panel:

    a. Demonstrate whether the biomarker panel can distinguish between drug-induced muscle injury and other causes of muscle injury.

    b. Provide a definition of threshold of injury (i.e., SKM biomarker levels X-fold above baseline levels signals clinically meaningful SKM injury/degeneration/necrosis in an individual subject).

    c. Demonstrate that the biomarker panel has the ability to predict clinically meaningful skeletal muscle injury, instead of simply to detect previous injury, and if the panel can be used to determine degree or severity of injury.

    d. Demonstrate that this biomarker panel is not elevated when injury has not occurred, or injury has occurred but resolved.

e. Provide a definition of sampling window (i.e., SKM biomarker is released into circulation immediately following drug-induced injury. After release into blood, its half-life is approximately X hours. The half-life should be used to guide the appropriate sampling time points when significant elevations of SKM biomarker are present).

f. Demonstrate whether the SKM biomarker panel is suitable for use with any developmental drug, regardless of class or mechanism that results in skeletal muscle injury/degeneration/necrosis.

g. Demonstrate whether the absence of increases in the SKM biomarker panel following drug exposure indicates absence of SKM injury.

h. Demonstrate whether the return of SKM biomarkers to baseline levels within the normal reference range after initial indication of injury (i.e. X-fold above baseline) indicates resolution of injury.

i. Demonstrate whether clinical trial participants should be restricted from moderate to heavy exercise for a specified time before sampling for the biomarker panel.

j. Demonstrate whether the normal reference range for individuals is (or is not) affected by age, sex, race, body weight, concomitant medications, tobacco/alcohol use, exercise, etc.

k. Demonstrate whether the biomarker panel will be able to indicate the presence of acute drug-induced myotoxicity earlier than standard methods (CK, AST).

l. Explain how differences in the four biomarkers (i.e., if only some are abnormal or if they are not consistent with CK elevations) will be interpreted. Additionally, clarify whether all four biomarkers carry the same clinical significance in evaluating muscle injury.

m. Provide a description of how the biomarkers will be used individually or as a panel.

n. Provide prospective clinical data with healthy patients in an early drug study and how these biomarkers can specifically identify drug-induced skeletal muscle injury

o. Provide guidance on the clinical significance of biomarker elevation; for example, whether the drug should be held, dose lowered, or drug discontinued based on different elevations in the biomarker panels.

9. Please explain how these biomarkers will be implemented broadly in drug development programs. One issue with these biomarkers is that the assays are research use only. You state that there are no standard operating procedures for sample collection, storage, or assay conduct established at this time, and assays will not be performed in a Clinical Laboratory Improvement Amendments certified laboratory. In contrast, assessments of CK and AST are low-cost assays and widely available.

10. Please submit a detailed statistical analysis plan for all the proposed clinical studies in your qualification plan. We may have additional comments at that time on your proposed statistical analyses. As discussed above, it will be important to establish the predictive ability of drug induced SKM injury of each of the biomarkers in the panel and the whole panel as intended to be used in clinical trials.

Please contact CDER BQP Program at CDER-BiomarkerQualificationProgram@fda.hhs.gov should you have any questions related to DDTBMQ000081. We look forward to working with you on this beneficial project.

Sincerely,

Christopher L. Leptak -S

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