DDTBMQ000050

October 25, 2018

Critical Path Institute
1730 E. River Rd.
Tucson, AZ 85718

Dear Dr. Sauer:

We are issuing this Transition Summary Response Letter to the Critical Path Institute's Predictive Safety Testing Consortium Hepatoxicity Working Group (CPATH PSTC-HWG), to notify you of our decision on 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 transition summary of May 22, 2018. We support and encourage your ongoing study to identify hepatocellular injury in healthy subjects and patients in conjunction with a panel of standard DILI biomarkers in all stages of drug development.

You have proposed qualification of a molecular safety biomarker to be used together with a panel of standard DILI biomarkers in clinical drug development trials for monitoring specifically for hepatocellular injury. 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 transition summary, we agree there is an unmet need and agree that development of the proposed safety biomarker would potentially enable detection of liver injury.

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 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.

In addition to the qualification effort, we encourage further study of your biomarker including collection of specified exploratory information from the proposed clinical trials. 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: *Glutamate dehydrogenase*

Type of Biomarker: Molecular
Matrix: Serum
Acronym: GLDH
Matrix: Serum
Uniprot ID: P00367

FDA's questions for continued development of the biomarker description: We agree with your description of the above biomarker.

Context of Use (COU) Considerations

Requestor's COU: *Serum enzymatic activity of glutamate dehydrogenase (GLDH) is a safety biomarker for monitoring specifically for hepatocellular injury in healthy subjects and patients in conjunction with a panel of standard D ILI biomarkers in all stages of drug development trials.*

Based on the review of the existing data on GLDH and the proposed protocols submitted by the sponsor, FDA has the following comments:
The COU for the serum biomarker, GLDH, will eventually depend on the quality and amount of data you submit with the FQP submission for our review. However, based on the review of your submission, we remain concerned that in patients with pre-existing liver injury, GLDH may not be able to distinguish worsening of underlying liver disease from drug induced liver injury. Currently, it appears that GLDH may be able to distinguish whether the source of injury is muscle or liver. Please conduct appropriate studies and submit adequate data to support the use of GLDH in the intended population.

To better understand the benefits of the identified biomarker as a DDT, and to continue to refine the COU, please provide the following information;

1. You indicated that the GLDH biomarker will be used in conjunction with a panel of standard drug-induced liver injury (DILI) biomarkers including alanine aminotransferase (ALT). You have also clarified that the novel biomarkers will be used in conjunction with other clinical and traditional biomarker data to identify the drug-induced liver injury in subjects with muscle impairment and for making treatment decisions on an individual subject basis in all stages of clinical trials. However,

you have not provided a detailed list of biomarkers included in the DILI panel. You have provided three rules related to when actions should be taken based on increases in GLDH or ALT as much as 2.5, 3, or 5 multiples of upper limit of normal (ULN). However, you did not provide a decision tree to guide the use of the novel biomarkers. GLDH along with the panel of DILI biomarkers. Please provide the list of biomarkers that will be used in conjunction with GLDH. Please provide your decision tree, and please rewrite your context of use to incorporate these concepts.

Analytical Considerations

2. Recovery

   On page 26 of the amended validation report in your transition summary (Appendix II), you provided summary data for the recovery study. In addition to the samples assayed previously (three samples of human serum spiked with concentrated calibrator and two samples of human serum spiked with patient LT-1041 serum), you added a 6th sample (human serum pool spiked with concentrated calibrator) near the 48 U/L medical decision level. In your table, the observed GLDH concentrations for the five samples previously measured are identical to the values reported in the Initial Briefing Package. However, the expected concentrations for these samples were different in the Initial Briefing Package (6.7, 13.5, 24.7, 128.9, and 302.1 U/L) and the % Recovery values calculated were different as well (113, 99, 84, 96, and 93%). It is unclear from the information you provided why these data would be different. If you analyzed all samples again in your response to FDA's comments on the Initial Briefing Package, please explain why the expected concentrations for the first five samples are different in the transition summary than in the Initial Briefing Package and why the observed concentrations for these five samples have not changed. If you only added data for the 6th sample, please provide a scientifically sound justification for why the data for the first five samples have changed. When submitting your QP submission, please submit a detailed protocol and data for a new recovery study for FDA review.

3. Interference

   Your transition summary does not include any of the additional information requested by FDA in feedback to the Initial Briefing Package in 2017, including a detailed protocol (describing, for example, the amount of GLDH in the samples), the raw data for the interference study, and a justification for your choice of acceptance criteria. Based on limited information available in the transition summary, it appears that only one high GLDH concentration was tested with varying levels of interferents and that interference was not assessed in samples with GLDH concentrations close to the medical decision levels. Therefore, this interference study is not yet informative. In addition, it is unclear from the study description how you would obtain robust %CV information from comparing just two samples with or without interferents. You have also not provided any information on how the relative error (%RE) is calculated. Please provide the requested information.

4. You have provided summary level methods and results in the transition summary. In your Qualification plan, please provide the full methods and results for your assay validation. Please also include the pre-analytical data in these reports. Please provide reports such as the calibration of the platform and other pre-analytical information for the equipment used to measure GLDH levels.

5. You state that blood samples from healthy patients and patients with evidence of liver disease were obtained from samples that would have been discarded. Please provide more information on the collection, processing, and storage of these samples. If samples were collected, processed or stored in different methods, please describe the different these different methods, and explain how these different methods do not affect the analysis of these samples. Please describe how it was determined that the patients had evidence of liver injury.

Clinical Considerations

6. GLDH is expressed in many organs (kidney, pancreas, gastrointestinal tract etc.), in addition to liver, and there is uncertainty whether these organs may exhibit an increased activity in disease states.

   You intend to use the GLDH as a biomarker in both healthy subjects as well as in patients with a wide range of disease states (including pre-existing liver disease). The proposed context of use (COU) is broad, thus the data generated from the proposed studies may not be sufficient to support adequate interpretation of results. The studies proposed are small, enrolling a limited number of patients, and in limited disease states, and such data may not provide accurate sensitivity, specificity, concordance, positive and negative predictive values.

   Based on the review of the four protocols submitted in the transition summary, you have not provided a plan that will allow adequate characterization of GLDH in different subgroups of patients across the full spectrum of disease severity. To adequately characterize GLDH, we recommend that you test GLDH performance at least in patients with moderate and severe diseases in all your proposed protocols. In addition, we have the following comments:

   a) In your QP submission, please discuss how your study will demonstrate whether the magnitude of GLDH elevation correlates with severity of liver injury.
   b) Describe the kinetics of serum GLDH in different patient populations, and whether certain factors can impact the kinetics. Specifically, will the kinetics be any different if patients have variable severity of pre-existing liver injury.
   c) Please assess and report the intra-patient variability of GLDH for the different age groups, genders, and other patient populations you plan to study. This intra-patient variability is needed to assess factors that can affect GLDH values and help establish a stronger reference range of GLDH values for these different patient populations.

7. Protocol specific comments:

   a. Protocol # 1- As currently proposed, you may not be able to enroll a sufficient number of patients with liver diseases to characterize GLDH across different disease etiologies, severities, gender, and age-ranges. The sample size may not allow satisfactory representation of specific liver diseases. You intend to enroll 200 patients with liver diseases with different etiologies:

      i. Hepatocellular or cholestatic disease,
      ii. Hepatocellular carcinoma,
      iii. Hepatic congestion,
      iv. Drug overdose.

   Additionally, the study will enroll cirrhotic and non-cirrhotic adults and children. The GLDH levels may vary by age and gender. Moreover, as the liver disease advances and the liver reserves diminish over time, GLDH activity may decrease. You may be unable to characterize GLDH adequately in different subgroups of patients with liver disease by enrolling only 200 patients.

   b. Protocol # 2-. We recommend that you enroll healthy subjects with muscle injury, patients with myositis, muscular disorders etc. such that each stratum is represented adequately.

   c. Protocol # 3- Pre-specify the number of patients that will be enrolled with specific types of gastrointestinal, pancreatic, and renal injury, as well as the disease severity. We recommend that you should have adequate representation of disease severity across the spectrum or at a minimum, enroll patients with moderate and severe disease. Enroll patients with different disease etiologies, for example, acute pancreatitis as well as acute-on-chronic pancreatitis; include a sufficient number of subjects to represent different age ranges.

   d. Protocol # 4- we recommend that you enroll subjects with a variable degree of liver injury secondary to APAP overdose.

d) Provide a decision tree and schedule of assessments in your submission regarding the timepoint(s) when GLDH will be assessed. Once the transaminase elevations are observed, do you plan to obtain serial serum GLDH levels on the subject?

e) Characterize GLDH performance in commonly occurring metabolic states such as fever, sepsis (moderate and severe disease spectrum), and dehydration.

f) High serum triglycerides interfered with GLDH testing, therefore, samples with high serum triglycerides were not analyzed; this should be specified in the context of use. In addition, we

recommend testing whether such interference may be observed in the presence of hyper/hypoglycemia or hyper/hypo natremia.

g) GLDH may not distinguish drug induced liver injury in the presence of pre-existing liver disease, as GLDH elevations may be observed secondary to progression of the underlying liver disease, acute liver disease flares (e.g., viral hepatitis, autoimmune hepatitis etc.) or acute hepatic congestion. You should further discuss with the FDA about the adequacy of studies to support such a COU.

h) You are conducting all the clinical studies at the University of Michigan, i.e., a single center trial. The data obtained from these studies may not be generalizable to subjects of different ethnicity, genetics and environment. The findings from these studies should be externally validated across different populations.

i) We recommend that you conduct studies in animal models to elucidate the kinetics of serum GLDH with various drugs that can potentially cause DILI. Currently, you are characterizing the kinetics of GLDH in the presence of acetaminophen overdose; however, it is unknown whether GLDH kinetics will follow a similar time course and magnitude of elevation, if DILI occurs with drugs other than APAP. There are animal models that you can utilize for these studies. These animal studies could help establish GLDH ranges for other drugs that have a potential for inducing DILI. If other animal studies are planned, submit the protocol for our review and describe how these studies will support the proposed COU.

Statistical Considerations

8. In the planned confirmation study of the performance of GLDH as a biomarker of liver injury, you reduced planned sample size from 400 healthy subjects and 400 subjects with liver injury to 200 per group. We are concerned that the reduced sample size in subject with liver injury may compromise your ability to establish concordance, sensitivity, and specificity of GLDH for each individual disease state (subjects with sever, moderate, or minor liver injury) and drug treatment. For the sample size estimation, you assumed a Beta (5,6) distribution of sensitivity and specificity values over the range of 87.5% to 100%. Note that your Beta-Binomial model is equivalent to the Binomial distribution with probability of success p=0.932 (approximately). You should provide some rationale, e.g. data from your previous studies that supported your prior belief, for applying Beta-Binomial approach and specifically for the parameter values used in Beta distribution.

9. One of the intended conditions of use for GLDH states that if GLDH is not elevated, then liver injury can be ruled out and diagnosis of alternative sources of ALT increases should be sought. The proposed target success criteria for sensitivity with a target point estimation of 90% and a 95% confidence lower bound of 0.85 or higher may not be sufficient. We suggest that you propose a more conservative target

success criteria for sensitivity of GLDH to detect a liver injury (i.e., a 95% confidence lower bound of 0.9 or higher).

10. For the study in humans with muscle injury, you reduced the sample size from 200 to 120 subjects. False positives rates assumed to have Beta (5,10) distribution over the range of 0% to 10%. Please clarify how parameters for Beta distribution were selected. The same comment pertains to the study in humans with pancreatic, gastrointestinal and kidney injuries.

11. Please study whether the linear relationship of ALT and GLDH could be changed by treatment used in patients and how the dependence could impact concordance, sensitivity, and specificity of GLDH as a biomarker in the drug development.

If you have questions, please contact Chris Leptak (christopher.leptak@fda.hhs.gov) through email. We look forward to working with you on this beneficial project.

Sincerely,

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