QUALIFICATION PLAN DETERMINATION

DDTBMQ000050

May 13, 2020

Critical Path Institute (C-Path) Predictive Safety Testing Consortium's (PSTC) Hepatocyte Working Group (HWG)
Attention: John-Michael Sauer, Ph.D.
Critical Path Institute
1730 E. River Rd.
Tucson, AZ 85718

Dear Dr. Sauer:

We have completed our review of the Qualification Plan (QP) for Drug Development Tool DDTBMQ000050 determined reviewable on November 14, 2019 by the CDER Biomarker Qualification Program (BQP), submitted under section 507 of the Federal Food, Drug, and Cosmetic Act.

The QP is for Glutamate Dehydrogenase (GLDH), a safety biomarker proposed for detecting drug-induced hepatocellular injury that can be used in clinical trials for subjects and patients with elevated serum transaminases due to muscle injury or degeneration. GLDH should be used in conjunction with standard hepatic injury monitoring biomarkers (e.g. total bilirubin, alanine aminotransferase (AST) and alkaline phosphatase).

FDA has completed its review and has agreed to **ACCEPT** your QP. In preparing to submit a Full Qualification Package (FQP), please ensure that the FQP submission addresses the scientific issues and the recommendations outlined below. A FQP should include all the data collected to support your biomarker and context of use. Please provide all data collected and full analysis of the data in accordance with the protocols and the statistical analysis plan you have provided in the QP. Please describe how your data and analyses have addressed knowledge gaps identified during the qualification process, and how the biomarker data support the context of use.

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

Biomarker: *Glutamate dehydrogenase* (GLDH)

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

Requester's Description: We agree with your biomarker description of glutamate dehydrogenase (GLDH). GLDH is a mitochondrial enzyme which is found in significant concentrations in the liver versus other organs including muscle tissue.

The proposed biomarker qualification will use the definition of hepatotoxicity which is met if one of the following statements is true:

1. ≥ 2.5x Upper limit of Normal (ULN) for GLDH and ≥ 2x (25 U/L) ULN for Total bilirubin (TBili)

2. ≥ 5x (48 U/L) ULN for GLDH, or ≥ 2x ULN for Alkaline phosphatase (ALP)

The reference range for GLDH in humans in a healthy volunteer population is < 3 to 10 U/L with an upper limit of normal (ULN) of 10 U/L. Currently, an elevation of alanine aminotransferase (ALT) greater than 3x or 5x ULN in addition to the other standard hepatic injury biomarkers listed above, are the accepted benchmark of concern for possible drug induced liver injury (DILI) in clinical trials. GLDH will be compared to ALT as used currently to assess hepatic injury.

Context of Use (COU) Considerations

Requestor's COU: *Serum glutamate dehydrogenase (GLDH) is a safety biomarker capable of detecting drug-induced hepatocellular injury that can be used in place of alanine aminotransferase (ALT) in clinical trials for subjects and patients with elevated serum transaminases due to muscle injury or degeneration. GLDH should be used in conjunction with standard hepatic injury monitoring biomarkers (e.g. total bilirubin and alkaline phosphatase).*

We have the following comments about COU considerations:

FDA Proposed COU: Serum glutamate dehydrogenase (GLDH) is a safety biomarker capable of detecting drug-induced hepatocellular injury that can be used in clinical trials for patients with elevated serum transaminases due to muscle injury or degeneration and with no pre-existing hepatic disease. GLDH should be used in conjunction with standard hepatic injury monitoring biomarkers (e.g. total bilirubin, ALT, and alkaline phosphatase).

You should address items 1 and 2 below in your FQP submission:

1. In your proposed COU, you state GLDH can be used in place of ALT in clinical trials for patients with elevated serum transaminases due to muscle injury or degeneration. ALT can be measured and should be measured in this population. Please revise your COU to include ALT as a standard hepatic injury biomarker. If you believe ALT should not be included in this COU, please provide reasoning for not measuring ALT in this population.

2. Your proposed COU does limit the patient population to patients with elevated transaminases due to muscle injury or degeneration. The COU should also be limited to patients with no pre-existing liver disease. Base on your completed and proposed studies, it does appear that GLDH is not well characterized in patients with pre-existing liver disease. Patients with pre-existing liver disease may have elevated GLDH levels which may not be due to drug induced liver injury and GLDH levels may not be able to discern drug induced liver injury in these patients. Additionally, there is limited information on performance of GLDH in cirrhotic population.

Analytical Considerations

We have the following comments about analytical considerations:

GLDH is measured by an assay that measures the enzymatic activity of GLDH. The activity is measured spectrophotometrically. The following performance characteristics were evaluated in the analytical validation report provided:

Limit of Blank/Limit of Detection: A deionized sample of water was tested to observe any reaction. After running 20 samples, the mean value of the runs was .1. The limit of detection test also ran 20 samples of a 10% solution of the calibrator (28 U/L) in deionized water. The Limit of detection was determined to be 2 U/L. Because the precision of the assay did not meet acceptance criteria (10% CV) at the limit of detection, the limit of detection was determined to be <3 U/L. The manufacturer's site states that the limit of detection for this assay is 2.9 U/L. These results are acceptable.

Precision: Precision testing was completed on four concentrations (2 U/L, 7 U/L, 53, U/L, 623 U/L) and two quality control samples. The requestor stated the acceptance criteria for this testing was 10%CV. Both within run and between run precision was assessed two times for 20 days. The 53 U/L concentration tested the precision of the assay near the proposed 5X ULN hepatoxicity value being used in the study. The

precision data demonstrated that the assay had less than 5% CV at 53 U/l and 623 U/L concentrations, and 13% CV at 7 U/L concentration. The precision at detection limit (2 U/L) was 37.7%CV. The precision was determined to be acceptable near the relevant proposed 53 U/L hepatoxicity level. The precision at 7 U/L was below the set acceptance criteria of 10% CV. This value is relevant because it tested the assay precision at the normal range of GLDH (<3-10 U/L). Upon examining the individual runs, the data indicated that all runs were within ±2 U/L. Because the %CV at this level would be more affected by changes in runs, the precision testing for this level was also found acceptable. Testing the precision around the other GLDH hepatoxicity value of 25 U/L or 2.5x ULN of GLDH would have also been beneficial. The precision testing did include precision testing using two quality control samples of 16U/L and 29 U/L. The %CV for these two samples was 8% and 4.7%. In a question below, additional information is requested on these quality control samples.

Method to Method comparison: This test evaluated the assay performance at 2 separate CLIA certified laboratories. 40 human samples were split and analyzed in two different labs. This testing provided limited data because the samples tested were small and 4 out of the 40 samples had a difference in measurement which was greater than 10%. The requestor conducted a regression analysis and found that the R value was .909. The study also provided information about site-to-site variability. This information did not affect the analytical validation or review of the assay.

Linearity: Two separate linearity studies were conducted on two human samples with elevated GLDH concentrations. The samples were diluted and measured at (1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256). Both studies demonstrated the assay linearity from 3 to 500 U/L. This linearity study is adequate.

Accuracy/Recovery: Because a commercially available standard material with GLDH was not available, a calibrator supplied by the manufacturer was used to create a sample with 110 U/L of GLDH. A human sample with high GLDH value and the calibrator were measured to confirm the values of these samples. The recovery was then conducted using 5 human samples which were spiked with either the calibrator or the high human sample.
In a second recovery test, a GLDH sample at 93 U/L was created using the same manufacturer calibrator. A human pool sample was then spiked with the calibrator. The recovery studies demonstrated that all samples were measured between 80-120% of the expected value. The recovery studies tested samples at the following expected values, spiking samples with the calibrator or high human sample (6.8 U/L, 13.3 U/L, 24.2 U/L, 137.8 U/L, 333.9 U/L and 46.5 U/L). In the questions below the requestor is asked to provide a justification why acceptance criteria of 80-120% recovery would not have a significant impact at clinically relevant GLDH concentrations.

Interference: Basic endogenous interference testing was provided in the analytical validation report. This assay was tested for interference from hemolysis, lipemia, and icterus. The interference testing was conducted by spiking a high GLDH pooled

human sample with interferent. The sponsor concluded from this testing that high levels of lipemia can affect the measurement of GLDH. The GLDH samples were significantly higher than the 2.5x or 5x upper limit of normal (ULN) proposed threshold for liver toxicity. The requestor stated that for these interferants the concentration of GLDH is irrelevant because it is more important to assess what will affect the light absorption during the testing. This conclusion does not address the assay performance around the clinically relevant levels that will be used to assess liver toxicity. It is unknown how lower levels of lipemia may affect the assay near the 2.5x and 5x the ULN for GLDH. The interference testing provided did not test exogenous interference or the potential for drug interference with the assay. In the questions below the requestor is asked to address these concerns with additional testing or information.

Freeze/Thaw Stability: Three different human samples of varied GLDH concentration were frozen at -80ºC and thawed 4 times. The samples were measured after each thaw cycle. All samples were measured with 86-103% of the true value. This Freeze/Thaw stability data is acceptable.

Sample Stability: Three different human samples at different concentrations were tested at baseline, 4, 24, 48, 72, and 96 hours for both room and refrigerated temperatures. The refrigerated samples were also tested at 1, 2, 4, 8, 10, 14, 21, and 28 days. Frozen samples were tested at 1 week, 2, weeks, 1 month, 3 months, 6 months, 12 months, and 18 months. The percent recovery for each storage timepoint was calculated relative to the baseline value. Most samples had appropriate percent recovery (80 120%); based upon the results of this testing and the acceptance criteria GLDH in human serum demonstrates acceptable stability for room temperature up to 48 hours, refrigerated up to 14 days, and frozen up to 18 months. This sample stability testing is acceptable.

You should address items 3-8 below in your FQP submission:

3. The composition of the quality control material used in the precision study is not clear. To evaluate whether the use of quality control materials is sufficient to determine precision of the GLDH assay, provide additional information about these materials, including the source of GLDH, the control material matrix, and value assignment protocols.

4. The analytical validation report for the interference study provide information for spiked hemoglobin, lipemia, and spiked totally bilirubin at GLDH concentrations significantly higher than clinically relevant levels. You also state that interference is independent of GLDH concentration because interference is caused by impending light transmission. It is unknown how lipemia or other components may interfere with the assay at the clinically relevant GLDH levels. In response to previous comments by FDA, you changed your definition of significant interference for this study to ±20% recovery. Please provide additional data or information about interference at clinically relevant

GLDH levels and explain why your revised acceptance criteria do not lead to a significant impact at clinically relevant GLDH concentrations.

5. It does not appear that you have considered other drug interference and exogenous interferences. Please conduct a risk analysis to identify potential drug interference and exogenous substances that may interfere with the assay.

6. You have defined acceptance criteria for several analytical studies, including your accuracy study, that appear to be broad (80-120%). You should provide information to show that the total analytical error of this measurement procedure (that is the cumulative effect that different sources of error, including bias or systematic differences as well as imprecision, allowable error from interference, allowable error from sample stability, etc.) would not have a significant impact on clinically relevant GLDH concentrations.

7. Please clarify if your current method measures GLDH from intact mitochondria or injured mitochondria. If your current methods include removal of intact mitochondria from injured hepatocytes (i.e. > 14,000 x *g* centrifugation of blood, instead of 3000 x *g*), then hepatotoxicants that do not cause mitochondrial dysfunction will not elicit a GLDH response.

8. Your submission states that all analytical testing is complete, and no further testing will be conducted. Your full analytical validation report for the GLDH assay included results for the following performance characteristics accuracy, precision, analytical sensitivity (limit of blank (LOB)), analytical specificity to include interfering substances, reportable range, reference interval, long term stability, and freeze/thaw stability. A summary of this testing is also provided in the executive summary. The current data provided do support moving forward with your proposed studies analysis. A final review of all data will be completed when the data and information are received in the full qualification package. If any additional testing or information become available, please provide the most up to date analytical validation in your Full Qualification Package and please address the points provided above.

Clinical Considerations

We have the following comments regarding clinical considerations:

The QP submission included data from completed studies for GLDH. This completed studies were exploratory and provided references for further studies for GLDH as a safety biomarker. The completed studies included establishing a GLDH reference range for healthy patients, correlated GLDH with ALT, and established that GLDH is not affected by muscle injury.

The QP provides plans for proposed studies on collected samples from patients. These studies include Confirming the Linear Relationship of ALT and GLDH (protocol

1). Confirming that GLDH Does Not Increase with Muscle Injury (protocol 2), Confirmation that GLDH Does Not Increase in Humans with Pancreatic, Gastrointestinal, or Kidney Injury (protocol 3), and Characterizing the Elimination Kinetics of GLDH and ALT in Humans (protocol 4). Based on the COU to use this liver safety biomarker in patients with muscle injury or degeneration in addition to standard hepatic injury monitoring biomarkers, the proposed studies appear to support the context of use which will provide data on this biomarker for this patient population. There are concerns that the patient samples to be analyzed are from one location, but the proposed study and analysis plans are acceptable.

You should address items 9-20 below in your FQP submission:

9. Transaminases (ALT and AST) are elevated when muscle injury occurs, therefore these enzyme measures can confound interpretation of liver injury (specifically DILI). In the biomarker interpretation section, you state that if GLDH is not elevated in subjects with muscle injury or degeneration then DILI can be eliminated. GLDH may have the potential to distinguish presence of DILI from muscle injury. While GLDH may have the potential to identify DILI, it is not necessary that GLDH elevations are observed every time liver injury occurs. For example, ~33% (6 of 18 subjects) patients with cirrhosis did not have elevated GLDH despite meeting the criteria for liver injury as assessed by ALT ≥3x ULN and TB ≥2x ULN. The performance of GLDH in patients with liver fibrosis and cirrhosis is unknown. Additionally, it is unknown whether there are conditions in which GLDH elevations may not occur despite presence of liver injury. Please revise this statement.

10. As suggested in the previous feedback letter dated October 25, 2018, you have planned to conduct additional nonclinical support for the use of GLDH to detect liver injury during concurrent muscle injury. In this study you plan to only use acetaminophen to assess liver injury and its effects on ALT, AST, CK and GLDH. Please also consider using other known hepatotoxic drugs to characterize GLDH in drug induced liver injury.

11. In protocol 1, please provide the type and severity of liver injury that will be included for the 200 patient samples that will be analyzed.

12. In protocol 2 you propose to confirm ALT 3x and 5x ULN correlation to GLDH 2.5x and 5x ULN. Please explain if these proposed correlations are always true or could different severity of injury affect this correlation.

13. In addition to Receiver Operating Characteristics curves, please provide sensitivity, specificity, positive predictive values, and negative predictive values comparison data for all the proposed studies.

14. In addition to the completed studies assessing GLDH, you propose 4 additional studies to support your proposed COU. Your proposed studies and analysis should demonstrate the data will support the COU. Please consider including data and analysis from literature, your completed studies, and results from your proposed studies in your effort to support the COU. A final review of all data will be completed when the data and information are received in the full qualification package.

    On February 6, 2020 a telephone conference was held between the FDA and CPATH. The following comments were emailed prior to the telephone conference and discussed during the telephone conference. Please ensure the data or additional literature address these comments which are provided below for your reference:

15. Please provide data or published papers which contain data for the description of the kinetics of GLDH in different patient populations, and whether certain factors can impact the kinetics. In protocol 1 “Confirmation of the Linear Relationship of ALT and GLDH in Humans”, and protocol 4 “Further Characterization of the Elimination Kinetics of GLDH and ALT in Humans”, please characterize the kinetics of GLDH in patients with varying degree (severity) of APAP induced liver injury. Additionally, blood samples should be collected at frequent intervals to accurately capture kinetics of GLDH relative to ALT. Please provide this sample collection interval data in your final qualification plan.

16. In the previous completed study “Establishing GLDH Reference Range”, you provide data on intra- and inter-patient variability for different age groups and racial groups. A majority of patients in this study were Caucasian. In protocol 1 “Confirmation of the Linear Relationship of ALT and GLDH in Humans”, and protocol 2 “Confirmation that GLDH Does Not Increase with Muscle Injury in Humans”, please assess and report the intra-patient variability of GLDH for the different age groups, gender, and other patient populations you plan to study. This intra-patient variability is needed to assess factors that can affect GLDH values and will establish a stronger reference range of GLDH values for these different patient populations. It is unclear if your proposed studies will address this comment. Please discuss how the proposed studies will include a diverse group of patients. Limiting the COU to patients with muscular injury does not address GLDH levels in different age groups and gender.

17. In Protocol 3, “Confirmation that GLDH does not increase in humans with Pancreatic, Gastrointestinal or Kidney injury”, please 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. Include 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.

18. Protocol 4 "Further Characterization of the Elimination Kinetics of GLDH and ALT in Humans", we recommend that you include subjects with a variable degree of liver injury secondary to APAP overdose.

19. Please provide any literature or information which characterizes GLDH performance in commonly occurring metabolic states such as fever, sepsis (moderate and severe disease spectrum), and dehydration.

20. 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 or as a consideration when using this biomarker. In addition, we recommend testing whether such interference may be observed in the presence of hyper/hypoglycemia or hyper/hyponatremia.

Statistical Considerations

We have the following comments regarding statistical considerations:

The original QP included descriptive summary results for the completed studies. In January 2020, the requestor was asked to provide patient-level data for the two completed studies: "Establishing GLDH Reference Range in Healthy Patients, and Establishing Sensitivity of GLDH and the Correlation of GLDH with ALT. The patient level data and descriptive statistics was found acceptable.

The requestor was also asked to provide statistical analysis plans (SAPs) for the following proposed studies: Confirming the Linear Relationship of ALT and GLDH in Humans (protocol 1), Confirming that GLDH Does Not Increase with Muscle Injury in Humans (protocol 2), and Confirmation that GLDH Does Not Increase in Humans with Pancreatic, Gastrointestinal, or Kidney Injury (protocol 3). In general, the SAPs were acceptable for the proposed study objectives.
However, please incorporate or provide the following additional information in the Final Qualification Package.

You should address items 21 and 22 below in your FQP submission:

21. Page 4 of the "Confirmation of the linear relationship of ALT and GLDH in humans" study SAP states "Summary measures as described in Section 3.2 will be conducted on etiology subgroups (e.g., acetaminophen overdose), to evaluate the consistency of the GLDH vs ALT relationship across those subgroups." Please pre-specify the subgroups that you will use in your analyses.

22. Regarding the "Confirmation of the linear relationship of ALT and GLDH in humans" study, we acknowledge your concern that ensuring sensitivities in the 91%-95% range would require a larger sample size. The target success criteria in your proposal

includes “Observed sensitivity ≥ 90%” and “95% Lower Confidence Bound (LCB) ≥ 85%”. In general, target success criteria should not rely on observed point estimates. Also, based on your proposal, the width of the confidence interval could be 10% or more. We encourage you to utilize a higher precision in the estimation of the three summary measures.

Please note that section 507 of the FD&C Act includes transparency provisions that apply to your submissions. Certain information contained within your submissions may be made publicly available on the Internet, as required by section 507. For examples of transparency and prior submissions see the Biomarker Qualification Submissions webpage[1].

If you have questions, please contact the CDER Biomarker Qualification Program at CDER-BiomarkerQualificationProgram@fda.hhs.gov should you have any questions (refer to DDTBMQ000050). We look forward to working with you on this beneficial project.

Sincerely,

Christopher L. Leptak -S

Digitally signed by Christopher L. Leptak -S
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1 https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificatio nProgram/ucm535881.htm

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