LETTER OF INTENT
DETERMINATION LETTER

DDTBMQ000100
August 28, 2020

University of Washington
Attention: Sean C. Murphy, MD, PhD
Department of Laboratory Medicine
Malaria Molecular Diagnostic Laboratory
750 Republican Street, F870
Seattle, Washington 98109, USA

Dear Dr. Murphy:

We are issuing this letter to University Of Washington Malaria Molecular Diagnostic Laboratory to notify you of our determination 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 Letter of Intent (LOI) deemed reviewable on March 10, 2020, and have concluded to **Accept** it into the CDER BQP.[1] Based on our review of the LOI, we agree there is an unmet need, and the development of this biomarker for monitoring patients in controlled CHMI studies may be helpful to reduce the time to initiate treatment and before the onset of malaria symptoms for drug and vaccine trials in endemic areas.

You have proposed *Plasmodium* 18S rRNA/rDNA as a monitoring biomarker that informs initiation of treatment with an anti-malarial drug >6 days following CHMI with *Plasmodium falciparum* sporozoites in healthy subjects (18-45 years old) from endemic areas enrolled in clinical studies for vaccine and/or drug development. As this biomarker development effort is refined in subsequent BQP submissions, the submitted data, the specifics of your context of use (including the target patient population), the specific analytics and the design of study(ies) used in the clinical validation of the biomarker will ultimately determine which of the comments below may be the most applicable to your qualification effort.

Your next stage of submission, a Qualification Plan (QP), should contain details of the analytical validation plan for the biomarker panel measurement method, detailed summaries of existing data that will support the biomarker panel and its context of use (COU), and include descriptions of knowledge gaps with proposed mitigation strategies. If future studies are planned, please include detailed study protocols and the statistical analysis plan for each study as part of your QP submission. Below, we provide you with specific considerations and recommendations to help improve your preparation for, and submission of the QP. For more information about your next submission and a QP Content Element outline, please see the BQP Resources for Biomarker

[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.

Requestors web page.[2]

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. We appreciate the complexity of the proposed endeavor and note its ambitious goals. However, we have several concerns related to the studies proposed by you and interpretability of potential results.

Biomarker Considerations

Requestor's Biomarker Description: *Plasmodium 18S rRNA/rDNA extracted from liquid whole blood samples or dried blood spots (DBS) followed by quantitative reverse transcription PCR (qRT-PCR).*

As in the previous malaria biomarker qualification DDTBMQ000044, qualified in October 2018, we agree *Plasmodium falciparum* 18S rRNA/rDNA measured in blood samples can be used to provide information on patients in drug development and vaccine trials for malaria. Your data should demonstrate that this biomarker can support your proposed COU.

Context of Use (COU) Considerations

Requestor's COU: A monitoring biomarker, that when positive, informs initiation of treatment with an anti-malarial drug >6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoites in healthy subjects (18-45 years old) from endemic areas enrolled in clinical studies for vaccine and/or drug development.

FDA's suggested COU for continued biomarker development: We agree with your proposed COU. The COU may be modified based on the data provided in the QP and Full Qualification Package.

Analytical Considerations

Pre-Analytical Sample Collection, Handling, Stability and Supporting Standard Operating Procedures

1. You have stated that data will be provided from ongoing clinical studies in endemic areas. It is unclear if these studies are using the same methods for sample collection, handling, and storage of samples. Please provide the operating procedures to collect and store samples for each study. If the studies do use different procedures, please explain how the

[2] https://www.fda.gov/drugs/cder-biomarker-qualification-program/resources-biomarker-requestors

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different procedures will not affect the samples and analysis of these samples.

Validation: Calibration, Controls, and Verification of Repeat Measures (Variability) and Demonstration of Capability for Full Parameter Range (Performance)

2. You state that the original biomarker detection assay data were provided in a previous and now complete biomarker qualification submission. These analytical data were found adequate for that qualification and COU. Please explain how the analytical data that were submitted in the past qualification submissions are adequate for the COU in the current submission. Additional analytical data may be needed for your COU in this submission.

3. You state that the assay was modified slightly from that reviewed under your prior submission DDTBMQ000044. Specify the parameters you have measured to support the comparability of the modified assay with the 3rd generation assay. Note that the data supporting the performance characteristics of the assay should be based on the version of the assay to be used for testing of clinical specimens. Appropriate positive and negative controls should be included. Clarify if you are planning to change the annealing temperature with this newly designed set of primers and probes, to reduce cross-reactivity, as indicated. Changes to the chemistry or annealing temperature of the RT-PCR assay may also change the assay's validation parameters, such as sensitivity and specificity. Plan to conduct and provide the results of a comparison between the previous and modified assay conditions in future submissions.

4. The previous biomarker qualification was for CHMI studies in healthy volunteers. We suggest that cross-reactivity with pathogens, especially protozoans that are likely to be present in the patient population in endemic areas where you intend to conduct the CHMI studies be measured.

5. Ensure that a standard curve is included each time clinical specimens are tested; this should include appropriate dilutions for testing including concentrations $<5.3x10^5$ copies/mL.

6. If you plan to use a threshold cutoff based on quantitative measurements of parasitemia by the modified PCR assay, the precision of the assay around the decision point should be determined.

7. On Page 4, you state that "...may also be possible to use appropriately designed 18S rDNA PCR-only assays (as is performed by several other CHMI centers)." Please note that our review will be based on the modified assay you intend to use for testing of clinical specimens and not any other assay(s).

8. We encourage you to submit the information on the analytical aspects of the assay for

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review prior to testing of clinical samples.

Confirmation of Transparency of Analytics Technical Parameters

9. Section 507 of the FD&C Act includes transparency provisions that apply to your submission. Analytical information about the assays, device, and software may be publicly posted if the biomarker is successfully qualified by the Agency. For example, you refer to non-public information for analytical specificity of the modified RT-PCR assay you intend to use. To ensure the biomarker can be used as a drug development tool by any interested party, please confirm technical parameters and other pertinent information about the assays, device, and software that may be made public. The biomarker qualification process does not endorse the use of any specific device, assay or software with a qualified biomarker.

Clinical Considerations

Background

10. In your QP submission, please provide the procedures and protocols for all the clinical studies that will be used to support your COU. Because differences in study design (e.g., population demographics, microbiologic epidemiology, underlying medical conditions) can limit the ability to draw meaningful conclusions from pooled data, please provide a detailed description of important differences in study design and an explanation for why these differences are not expected to impact the conclusions of your pooled analyses. Please explain why any differences between clinical studies will not affect the data between the studies.

11. In the LOI submission, you state the biomarker is primarily intended for use as a safety endpoint. However, the biomarker COU defines the biomarker as a monitoring biomarker used to determine when to initiate treatment in CHMI initiated infections. Therefore, in your QP submission, the protocols and plans for qualification should produce data to support the monitoring COU, and not other uses such as a safety endpoint.

12. It is unclear how the biomarker can be used pre-CHMI under the proposed COU, since the qualification is for monitoring of infection *after* challenge. Please explain how testing patients before initiation into the clinical trial is within the scope of the COU.

13. It appears that endemic subjects who are biomarker positive prior to CHMI were enrolled and that if the biomarker remained positive post treatment, other tools such as Sanger sequencing and next generation sequencing were used to differentiate the CHMI strain parasite from the field strain. It would be helpful if you could perform subset analysis for

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the patients that tested positive and negative by your RT-PCR assay, prior to CHMI. Please clarify if PCR testing was based on testing of single or two consecutive blood samples, collected on different days, prior to CHMI.

14. You intend to compare available biomarker data from outside laboratories to perform discrepant analysis. Clarify, whether the PCR assay is the same as the modified 3rd generation assay you intend to use for BQ. As stated above, the focus of our review will be the modified RT-PCR assay you intend to use. If testing is done in different laboratories using the same assay, then appropriate quality control measures should be implemented and the data supporting comparability of performance of the assay provided for our review.

15. You intend to provide data from peer-reviewed literature to support clinical validation. Please clarify if the data from the published studies will be based on the assay you have developed for BQ or different assay(s). Note that the focus of our review will be the modified RT-PCR assay you intend to use for testing of clinical specimens from endemic subjects enrolled in CHMI studies.

16. You list several studies in Table 1 of the LOI. Although you state on Page 7 that the inclusion/exclusion criteria are not significantly different from those used for the CHMI studies in non-endemic sites as submitted in Appendix 2 of DDTBMQ000044, it will aid in our review if all differences in the enrollment criteria between the studies in the endemic vs nonendemic subjects are provided.

17. Specify the strain of *P. falciparum* used for challenge and whether CHMI was induced by mosquito bites and/or sporozoite inoculation. All details of the methods such as preparation of infected mosquitoes or sporozoites including the name and address of the facility that was used as well as *in vitro* sensitivity to antimalarial drugs used as rescue therapy should be provided.

18. You intend to provide the results of vaccine trials to support the proposed COU. As the administration of vaccine(s) is likely to boost immune responses, please discuss in your QP whether the assay performance and the rate of sub-patent infections is likely to be different in the vaccine vs drug trials. We encourage you to provide data for the drug trials, in addition to vaccine trials; consider providing protocols for our review prior to initiation of the studies.

19. You state on Page 3 that “In addition, the biomarker may also detect sub patent CHMI infections that emerge from the liver into the blood but do not achieve a patent parasite density. Such infections can be suppressed by anti-erythrocyte stage antibody responses acquired by persons in endemic regions due to repeated natural exposures to *Plasmodium* parasites. Biomarker detection of subpatent infections may demonstrate that CHMI procedures were successful even if pre-existing immune responses subsequently modify

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the growth of such infections." Comment on whether differences in different endemic areas with high and low transmission rate will affect the performance of the biomarker such as thresholds due to differences in immune status of the hosts.

Interpretive Criteria (Cut-offs/Boundaries), Application & Validation in population

20. The COU states that initiation of treatment with an anti-malarial drug should occur 6 days after controlled malaria infection. Please provide data that confirms patients are biomarker positive 6 days after the controlled malaria infection. If patients are positive before this six-day period, please explain what action should occur.

21. On Page 5 you state that "This means that investigators who use the biomarker would specify in their protocol a biomarker-based definition that would be used to initiate treatment. This threshold may be different for studies with different types of products or different reasons for doing the study. Usually this threshold would be selected to reduce symptoms and accelerate infection detection compared to thick blood smear (TBS). For instance, the protocol could be one positive biomarker result over a defined threshold (e.g., $>1.85x10^6$ copies/mL, equivalent to ~250 estimated *P. falciparum* parasites/mL)." Please clarify, whether the data supporting the proposed threshold(s) in specific protocols will be provided as part of regulatory submissions such as an IND application.

Statistical Considerations

22. On page 7 of the LOI regarding how the biomarker measurement will inform drug development, it states this is primarily a safety endpoint, but the biomarker may be useful for efficacy by determining if a *Plasmodium* infection was present in the blood. This differentiation of a safety and efficacy endpoint is not clear. Please provide data and information that supports the proposed COU for this submission.

23. On page 7, the LOI lists how the biomarker measurement will inform drug development. Given that this qualification will be used at endemic sites, an important use will be to make sure that subjects are not infected prior to the start of the trial. This should be added to the list as well.

24. The decision tree states how the treatment threshold for the biomarker would be study-specific. However, it is not clear why the threshold for the determination of an infection will vary or how the choice will be made. Additional rationale for this varying threshold should be given.

25. Table 1 lists an endpoint of qPCR in 7 of the studies. Please clarify if any study used the UW *Plasmodium* 18S rRNA qRT-PCR.

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26. Clarify from which studies you have obtained blood samples. Of those studies, will you plan to assess all samples or a subset of samples (as implied on page 9). If you only plan to assess a subset of samples, explain the rationale for only testing a subsample and how you will choose the sample.

27. On page 9 of the LOI, it states that additional analyses may be undertaken to address issues more likely in endemic settings. It is not clear why you state that they "may" occur. We think that these analyses will be important for the qualification in the endemic setting.

28. Measures of sensitivity and specificity are useful in understanding the properties of diagnostic tests. The LOI states that the biomarker is more sensitive than the gold standard, TBS. Although we acknowledge that these conclusions may be reasonable if the results for each subject are concordant and positivity occurs with PCR prior to TBS, please clarify how you will interpret discordant results with regards to defining the sensitivity and specificity of your assay.

29. Although you indicate that the thresholds for future use of the biomarker will be study-specific, we recommend that you present the analysis results by a few thresholds to show the performance of the biomarker.

30. Please submit the statistical analysis plan in view of the above statistical comments.

Please address each of the specific considerations and recommendations and any data requests cross-referencing the numbered list above in a separate addendum to 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 and CBER divisions. 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. Sean Murphy (murphysc@uw.edu), the primary point of contact for this project.

Should you have any questions or if you would like a teleconference to clarify the content of this letter, please contact the CDER Biomarker Qualification Program via email at CDER-BiomarkerQualificationProgram@fda.hhs.gov with reference to DDTBMQ000100 in the subject line. For additional information and guidance on the BQP please see the program's web pages at the link below.[3]

[3] https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/cder-biomarker-qualification-program

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Sincerely,

Christopher L. Leptak -S

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