LETTER OF INTENT
DETERMINATION LETTER

DDTBMQ000107
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 May 18, 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 a biomarker endpoint for prophylactic efficacy trials of *P. falciparum* drug and vaccine trials in endemic areas would be beneficial because of the ability to assess parasitemia earlier than current use of parasite count on thick blood smear (TBS).

You have proposed *Plasmodium* 18S rRNA/rDNA as a surrogate endpoint biomarker to make efficacy determinations in *P. falciparum* prophylactic efficacy trial of drugs, vaccines, or other therapeutics in endemic areas. The concentration of biomarker required for surrogate endpoint determinations will be made on a trial-specific basis and defined in each clinical trial protocol As this biomarker development effort is refined in subsequent BQP submissions, the submitted data, the specifics of your context of use (COU), 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.

Context of Use (COU) Considerations

Requestor's COU: A monitoring and surrogate endpoint biomarker that indicates the status of infection in *Plasmodium falciparum* field prophylactic efficacy trials of drugs, vaccines, or other therapeutics in enrolled subjects in endemic areas. When used as a monitoring biomarker, this biomarker may be used to inform the initiation of treatment with an approved anti-malarial drug. The presence of the biomarker may also be used as a surrogate endpoint to make efficacy determinations in *Plasmodium falciparum* prophylactic efficacy trial of drugs, vaccines, or other therapeutics in endemic areas. The concentration of biomarker required for monitoring and/or surrogate endpoint determinations will be made on a trial-specific basis and defined in each clinical trial protocol.

FDA Recommended COU: Biomarker endpoint to be used in clinical trials to evaluate drugs and/or vaccines intended to treat or prevent *Plasmodium falciparum* in endemic areas.

1. Your COU statement includes two different uses (monitoring and surrogate endpoint) for your biomarker. Only one COU should be provided in a biomarker qualification submission. You should provide the COU that is the subject of the biomarker submission. Other COU statements for other projects, or qualified biomarker COU do not need to be provided in this qualification submission.

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

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2. For a biomarker endpoint COU, depending on the type of therapeutic or prophylactic product and the clinical context, additional clinical information may be needed to support a labeling claim or product approval. As your biomarker development effort continues, we will work with you to better define the type of biomarker endpoint COU and the use conditions for which it would be appropriate.

3. It appears that results for vaccine trials will be provided to support the proposed COU. As the administration of vaccine(s) is likely to boost immune response(s), please discuss in your QP, whether the assay performance and rate of sub-patent infections is likely to differ in the vaccine vs drug trials. We encourage you to provide data for the drug trials, in addition to vaccine trials. Please provide the clinical trial protocols in your future QP submission for our review prior to initiation of the studies.

4. We encourage you to collect and store samples for the field prophylaxis trials to support the intended COU. However, we recommend that the testing be performed after the results of the trials in endemic CHMI have been reviewed.

Analytical Considerations

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

5. 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 sample collection, handling, and storage of samples. Please provide the operating procedure to collect and store samples for each study. If the studies do use different procedures, please explain how the 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)

6. 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.

7. 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

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the assay to be used for testing of clinical specimens. Appropriate positive and negative controls should be included. Please 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.

8. 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 prophylactic field studies be measured.

9. On page 5 you state that "It may also be possible to fulfill the COU when using appropriately-designed 18S rDNA PCR-only assays (as is performed by several other CHMI centers)." Please note that our review will be based on analysis of the modified RT-PCR assay you intend to use for testing of clinical specimens and not on any other assay(s).

10. On Page 7 you state that "Data showing substantial equivalence between the revised assay and that reviewed under DDTBMQ000044 will be submitted for this endemic CHMI COU. DBS validation will cover the same topics to ensure reliable testing for the DBS sample type." Clarify what all parameters will be measured to support substantial equivalence.

11. Please 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.

12. 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.

13. We encourage you to submit the information on the analytical aspects of the assay for review prior to testing of clinical specimens.

Confirmation of Transparency of Analytics Technical Parameters

14. 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

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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

15. You state the limit of detection of the assay is $5.3x10^4$ copies/mL or 20 parasites/mL. You also state that subjects in an endemic areas may have a pre-existing infection. Because your study and assay provide quantified data, please explain how the results or analysis would be affected if a person were to have the biomarker below the limits of detection of the assay.

16. You state that the parasite density conversion factor was discussed in your prior biomarker qualification effort (DDTBMQ000044). Please clarify (and if so quantify) whether the conversion factor might be affected by the endemicity of malaria in the study regions as well as the modification of the assay.

17. Given that all the studies where samples will be collected and analyzed are from Africa, please discuss whether health factors such as sickle cell trait could affect the results of your study. Please indicate if this information will be collected and considered when analyzing the samples.

18. Please discuss in your QP whether the differences in the immune status of the patients in endemic areas with high vs low transmission rates are likely to alter the rate of sub-patent infections and likely to differ in the vaccine vs drug trials.

19. You do not intend to stipulate a specific treatment threshold for biomarker-detected infections. On page 5 you state that “This is consistent with the practice in the now-qualified non-endemic CHMI COU DDTBMQ000044 and the proposed endemic CHMI COU LOI DDT000100. In practice, if the biomarker was used to judge efficacy while TBS, RDTs, and symptoms were used to monitor participant safety, then criteria for interpretation of the biomarker-based infection detection would be listed in the trial protocol. Alternatively, if the biomarker was used at the endemic site in real time to monitor safety, the site would need to indicate a biomarker-based treatment threshold that would trigger treatment (akin to the situation in CHMI studies). Such a threshold would be indicated in the clinical trial protocol as well and allows for trial-specific tailoring of the

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exact biomarker cutoff for treatment. This field study COU is intended to qualify the biomarker as a true indicator of infection." Additionally, on page 6 you state, "When used as a monitoring biomarker, this biomarker may be used to inform the initiation of treatment with an approved anti-malarial drug. The presence of the biomarker may also be used as a surrogate endpoint to make efficacy determinations in *P. falciparum* prophylactic efficacy trial of drugs, vaccines, or other therapeutics in endemic areas." Please note that if your biomarker was qualified, we expect that the data supporting the threshold(s) to be used in clinical trial(s), to evaluate efficacy or safety, will be part of the regulatory submissions, such as an IND. We anticipate that the supporting data will be reviewed by the FDA prior to initiation of the phase 3 clinical trial(s).

20. On Page 7 you state that "There is excellent precedent for qualification of this biomarker for endemic prophylactic efficacy (non-CHMI) studies of preventive drugs and vaccines. To date, clinicaltrials.gov lists the following trials in malaria-endemic sites that have used TBS and *Plasmodium* 18S rRNA/rDNA biomarker-based endpoints (Table 1). A subset of these endemic site trials will be included in the Biomarker Qualification for the proposed COU." Clarify if the assay(s) are the same as the modified RT-PCR assay you have developed for this BQ. 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 in the prophylaxis trials. Please also see comments above regarding analyses for inter-assay variability, if applicable.

21. On Page 10 you state that "We will also compare to available biomarker data from outside laboratories (where available) to perform discrepant analysis in situations that are qRT-PCR biomarker-positive/TBS-negative." Please, clarify if the data from the published studies will be based on the modified RT-PCR 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 for prophylaxis studies.

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

22. You state a specific treatment threshold to determine efficacy will not be proposed in this qualification effort and that each clinical study should determine the necessary threshold for that specific study. It is unclear how your studies will demonstrate that this biomarker can assess efficacy of vaccines and drugs if no threshold value is used during the qualification process. While future clinical studies can set a specific threshold, for this qualification effort please describe how efficacy will be determined using the proposed biomarker.

Gaps and Proposed Studies

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23. It is unclear how the conversion factor to estimate parasite density is applicable to the COU in this submission. Please explain how the parasite density conversion will be used for a biomarker endpoint. In addition, it is unclear if this conversion factor can be used in patients who have a pre-existing infection. Please provide more information on this conversion factor for the COU and patient population in this current submission. In your QP submission, please include a detailed plan to compare severity of infection, clinical symptoms, and parasite density in your final analysis.

Statistical Considerations

In your qualification plan, please submit the statistical analysis plan (SAP) to support the proposed biomarker endpoint. Specifically, please provide a rationale for the utility of using a biomarker endpoint, its relationship to clinical information, and when additional clinical information may be needed. What is the primary statistical analysis method you plan to use to assess the presence of the biomarker that may be used to make efficacy determinations in *P. falciparum* prophylactic efficacy trial of drugs, vaccines, or other therapeutics in endemic areas? What statistical criteria will be the basis for establishing the performance goal of the proposed biomarker endpoint COU? How will the concentration thresholds of the biomarker be determined in the statistical analysis, etc.? If the above study design elements and statistical criteria are study specific, please include study-specific details in the SAP. Please also clarify the following issues.

24. You mentioned, “A subset of these endemic site trials will be included in the Biomarker Qualification for the proposed COU” (pp 7). If you only plan to assess a subset of trials, explain how you will choose the subset. Will you plan to assess all samples or a subset of samples from a trial. 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.

25. On page 8, 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. Should that be listed as a COU as well? Though, it may not appear to be directly related to the COU, it is not clear how determination of infection after treatment can be understood without a clear understanding of a subject’s status at baseline.

26. You mentioned “since the vaccine and drug products may vary, exactly how the biomarker will be used to inform prophylactic efficacy endpoints would be contained in each clinical trial protocol.” (pp 8) You should address how this will impact the use of the vaccine trials listed in table 1 to qualify the biomarker for use in drug trials.

27. 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

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be given.

28. On page 10 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 and ask that you describe the additional analyses, as well as rationale, in detail.

29. Measures of sensitivity and specificity are useful in understanding the properties of diagnostic tests with the gold standard clearly defined. Please provide details regarding how you plan to assess sensitivity and specificity if these measures are relevant for use of a biomarker endpoint.

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 DDTBMQ000107 in the subject line. For additional information and guidance on the BQP please see the program's web pages at the link below.[3]

Sincerely,

Christopher L. Leptak -S

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

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