REVIEW MEMO

Topic: *Plasmodium falciparum* 18s rRNA/rDNA gene, A-Type Biomarker Qualification

Materials Reviewed: Final Biomarker Qualification Package, dated March 12, 2017

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Sponsor: University of Washington, Seattle

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1. Background:

Historically, two species of malaria parasites, *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv), which infect humans, are used in deliberate controlled human infection for- (i) treating neurosyphilis ('Malaria Therapy') in early 1900s (In 1927 Julius Wagner-Jauregg won Nobel Prize in Physiology & Medicine for the treatment of dementia paralytica by malaria inoculation) and (ii) early phase malaria vaccine and drug development [e.g. prophylactic efficacy study of anti-malaria drug Paludrin (Bull WHO (1948), 192: 197-200)] studies. To date, detection of parasites under a microscope in the Giemsa stained thick blood smear (TBS) remain the 'gold standard' for parasite detection after controlled infection with sporozoites or blood-stage parasites. TBS has low sensitivity; it can detect a parasite in blood only after day 10 or 11 post controlled infection with sporozoites and most of the study subjects develop clinical malaria by then. In addition, quality control of TBS is a major issue as it depends on quality of the blood smears on slide, optimal staining, quality and training of microscopists etc. Therefore, a more sensitive parasite detection technique like quantitative RT-PCR (qRT-PCR) which is easier to standardize and validate across multiple centers, is desirable to support clinical trials designed for testing efficacy of newer anti-malaria drug and vaccine development efforts in which controlled human malaria infection (CHMI) will be a component. These sensitive parasite detection techniques help to detect parasites earlier than TBS and can facilitate initiating anti-malarial therapy in study subjects before clinical malaria sets in. RT-PCR has a potentially better scope for being the 'assay of choice' over PCR because it uses RNA to start with instead of genomic DNA and that can persist longer in circulation even after actual destruction of parasite due to effect of drug or vaccine and that will give 'false negative' efficacy result and 'false positive' safety result about the drug or vaccine in the study.

Over the years various PCR, Nested PCR and RT-PCR have been developed by different investigators with varied sensitivity and specificity. Majority of these assays were developed using the 18srRNA/rDNA sequence of the parasite as target of detection. However, none of those tests has ever been used as the primary assay to define the efficacy of an investigational malaria drug or vaccine. In recent years, many of the malaria vaccine development studies have used this as an additional tool alongside TBS, which has been by-and-large the primary mode of parasite detection in all those trials. In all of those studies, the common result which is observed is that NAT based techniques can detect parasites in blood 3-4 days (average) earlier than that by TBS and this can provide an opportunity to initiate early treatment of the study subjects before even they develop clinical malaria symptoms.

2. The proposed Context of Use (CoU), mentioned in the final package:

"Detection of the *P. falciparum* 18S rRNA/rDNA is a safety (and efficacy) endpoint for initiating treatment before clinical malaria symptoms appear in subjects who have undergone *P. falciparum* sporozoite CHMI in non-endemic regions. The biomarker can be tested for at ≥6 days post-CHMI in human whole blood. The *P. falciparum* 18S rRNA/rDNA biomarker must have been measured with one or more specific nucleic acid amplification-based methods. This biomarker is intended to replace the use of thick blood smear (TBS) microscopy for this endpoint."

Reviewers' Comments:

Upon review of the initial biomarker qualification package, we requested for a clarification whether the threshold for biomarker detection and parasite density estimation will be same for both efficacy and the safety (which is the decision point for initiating rescue drug treatment for the subjects) endpoints of 'novel' malaria vaccines and drugs under study. For example, if the protocol specifies the safety endpoint (i.e., the trigger to start rescue anti-malarial drug treatment) is 20 estimated parasites/µL blood on day 7 post-CHMI, will the efficacy endpoint of the novel malaria vaccine or drug under the study be set at ≤ 20 estimated parasites/µL on Day 7 post-CHMI too? In other words, if they see estimated parasitemias ≥20 parasites/µL, will they consider that novel vaccine or drug inefficacious? If they start rescue treatment at 20 estimated parasites/µL on Day 7 post-CHMI but their efficacy endpoint for the novel malaria vaccine or drug under the study is specified at ≥ 20 estimated parasites/µL, that will be outside of the scope of this biomarker qualification. From that perspective, we requested them to limit the scope of this biomarker qualification only to safety endpoint for starting rescue treatment early before clinical malaria symptoms appear in subjects post-CHMI using P. falciparum sporozoites.

<u>In the final package, the sponsor did not address the above issue</u>. Therefore, the proposed CoU is inappropriate and as we mentioned to the sponsor earlier, 'efficacy endpoint' is out of the scope for this qualification.

The appropriate CoU, based on the information provided in the final qualification package should be: *P. falciparum 18srRNA/rDNA gene, A-Type is a monitoring biomarker to inform initiation of rescue treatment with an anti-malarial drug following controlled human malaria infection (CHMI) with P. falciparum*

sporozoites in healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against P. falciparum.

3. Proposed Biomarker Assay [Detection] Characteristics:

The proposed biomarker assay used for this submission was a quantitative reverse transcription PCR (RT-PCR) assay. Analytical performance characteristics were assessed and are included herein. Assay characteristics include *P. falciparum*-specific and pan-*Plasmodium* detection using multiplexed reagents, analytical limit of detection (LoD) of 20 parasites/mL, precision across a wide reportable range that includes expected post-CHMI parasite densities, sample stability, information on laboratory interferences and workflow compatibility with same-day reporting for CHMI studies.

Reviewers' Comments:

RT-PCR is appropriate assay for detection of the biomarker as clarified in the Background section of this memo. But whether 'quantitative' RT-PCR is appropriate in comparison to simple RT-PCR is debatable. On one hand, the quantitation part is important to estimate number of parasites per certain density of whole blood, which in turn, perhaps, helps in having a better estimate for Limit of Detection (LOD) of the assay, but on the other hand, from clinical perspective, if that quantitation is to determine a treatment decision, it can be problematic. The good thing about almost all clinical study protocols which involve CHMI with P. falciparum sporozoites is that all subjects, irrespective of parasitemia status post-CHMI get anti-malarial drug treatment at the end of the study, typically 28 days post-CHMI.

Regarding pan-Plasmodium detection information included in the package, upon review of the Letter of Intent (LOI) package, the sponsor was informed that we consider the context of use for this biomarker is limited only to controlled human malaria infections (CHMIs) with P. falciparum sporozoites to support anti-malaria drug and vaccine development. Expansion of usage for this biomarker to detect parasite in field and epidemiologic studies falls largely in the realm of licensure of a molecular diagnostic for malaria and is outside the scope of biomarker qualification process. In that context, we advised the sponsor not to include in the final submission information on Pan-Plasmodium detection or detection of P. falciparum from mixed Plasmodium sp. infection as the possibility of using mixed species of Plasmodium in any CHMI study is very limited. The sponsor included that information in the final package.

In addition, the data in the package showed that a pan-Plasmodium test can also detect Babesia microti, another apicomplexan parasite, closely related to malaria and endemic in many upper North-Eastern and Central states of the USA and also Europe. Although B. microti is detected at a higher qRT-PCR cycle (Figure 13), all other Plasmodium species were detected almost at the same cycle level, using pan-Plasmodium specific reagents (Figure 12). Therefore, caution is required regarding possibility of false positive results if the proposed assay is conducted on subjects from the areas with known B. microti transmission.

4. Clinical information in clinical trials for this BQ:

The qualification package includes information regarding detection of the biomarker in stored blood samples of the study subjects from three retrospective clinical studies:

	MC-001	MC-003	PfSPZ-CVac PYR
Study	single-center, open-label Phase 1 trial to demonstrate mosquito bite CHMI under an IND and obtain immunological endpoints after a single exposure. The study utilized the five mosquito bite model.	single center, randomized, partially double-blinded, placebo-controlled Phase 1 trial of Infection-Treatment-Vaccination (ITV) using CQ with or without primaquine (PQ). The study utilized the five mosquito bite model.	single center Phase 1 study of ITV consisting of wild-type, aseptic, purified, cryopreserved *P. falciparum* sporozoites (Sanaria® PfSPZ Challenge) administered by Direct Venous Injection (DVI) in conjunction with Chloroquine and pyrimethamine (PYR).
Subjects	Healthy, malaria-naïve adult subjects were enrolled at the non-endemic site in Seattle	Healthy, malaria-naïve adult subjects were enrolled at the non-endemic site in Seattle	Healthy, malaria-naïve adult subjects were enrolled at the non-endemic site in Maryland
CHMI	5 infected mosquito bites	5 infected mosquito bites	3200 sporozoites through DVI
TBS Positive post-CHMI	average 11.2 days (95%CI 9.5 to 12.8 days)]	<u>For infectivity controls</u>, 6/6 subjects, average 8.7 days (95%CI: 7.6 to 9.8 days) <u>For immunized subjects</u>, 20/23 subjects, average 11.0 days (95%CI: 10.0 to 11.9 days)	[Total 21 (16 immunized group and 5 infectivity control group) subjects underwent CHMI. 6/21 immunized group subjects were protected (TBS negative/NIH PCR negative) upon CHMI. 15/21 subjects met the protocol-defined criteria for treatment (TBS-

			positive and/or NIH PCR-positive) and stratified as "TBS positive" and "All Treated (6 TBS positive/NIH PCR positive and 9 TBS negative (?)/NIH PCR positive)" TBS positive Group (n=6; 4/11 subjects from 2 immunized groups and 2/5 infectivity control group): average 13.5 days (95%CI: 11.0 to 16.0 days) All Treated Group (n=15; 6 subjects from TBS group + 9 subjects who were NIH PCR positive): average 12.0 days (95%CI: 10.8 to 13.2 days)
Biomarker Positive post-CHMI	average 7.7 days (95%CI 6.4 to 8.9 days)	<u>For infectivity controls,</u> 6/6 subjects, average 7.0 days (95%CI 7.0-7.0) <u>For immunized subjects</u>, 20/23 subjects, average 7.3 days (95%CI 6.8 to 7.8)	TBS positive Group (n=6; 4/11 subjects from 2 immunized groups and 2/5 infectivity control group): average 9.2 days (95%CI: 6.1 to 12.2 days) All Treated Group (n=15; 6 subjects from TBS group + 9 subjects who were NIH PCR positive): average 8.6 days (95%CI: 7.6 to 9.6 days)
			Grade 1: [TBS positive Group (n=6; 4/11 subjects from 2 immunized

Average days for appearance of Clinical malaria symptoms post-CHMI	Grade 1: 9.7 days (95%CI 6.4 to 13.0 days) Grade 2: 12.2 days (95%CI 10.6 to 13.8 days).	Grade 1: <u>For infectivity controls</u>, 8.0 days (95%CI: 6.7 to 9.3 days) <u>For immunized subjects</u>, 9.5 days post-CHMI (95%CI: 8.6 to 10.5 days) Grade 2: <u>For infectivity controls</u>, 8.8 days (95%CI: 8.2 to 9.4 days) <u>For immunized subjects</u>, 10.0 days (95%CI: 8.5 to 11.5 days)	groups and 2/5 infectivity control group)]: average 12.8 days (95%CI: 10.4 to 15.1 days) All Treated Group (n=15; 6 subjects from TBS group + 9 subjects who were NIH PCR positive): average 11.3 days (95%CI: 9.6 to 13.0 days) Grade 2: [TBS positive Group (n=6; 4/11 subjects from 2 immunized groups and 2/5 infectivity control group)]: average 13.8 days (95%CI: 12.2 to 15.3 days) All Treated Group (n=15; 6 subjects from TBS group + 9 subjects who were NIH PCR positive): average 13.5 days (95%CI: 11.9 to 15.1 days)
Accelerated parasite detection post-CHMI using Biomarker in comparison to TBS	3.5 days	<u>For infectivity controls</u>, 1.7 days <u>For immunized subjects</u>, 3.6 days	<u>For TBS positive Group (n=6; 4/11 subjects from 2 immunized groups and 2/5 infectivity control group)</u>, 4.3 days

Reviewers' Comments:

Information from three clinical studies included in the package establishes that the proposed biomarker is useful in detecting parasite well in advance of the current 'gold standard' assay, Giemsa stained Thick Blood Smear (TBS), and the onset of clinical

symptoms of acute malaria in malaria-naïve subjects who underwent CHMI using P. falciparum sporozoites. This has two implications: First, in early parasite detection before onset of clinical malaria will facilitate initiation of rescue treatment of the study subjects with anti-malarial drugs and thus onset of acute malaria in these subjects can be avoided. This will encourage more subjects to participate in clinical studies aimed at novel malaria vaccine and drug development. Secondly, as early detection of parasite through biomarker will avoid onset of acute clinical malaria in study subjects, it will reduce the cost of conducting clinical studies which involve CHMI by allowing the studies to be conducted without the current requirement and practice of putting the study subjects in confinement (e.g., hotel) post-CHMI for several days.

5. Recommendation:

I recommend approval of this biomarker qualification application.

Comments for the sponsor:

(i) Please modify the Context of Use (CoU) to: *P. falciparum 18srRNA/rDNA gene, A-Type is a monitoring biomarker to inform initiation of rescue treatment with an anti-malarial drug following controlled human malaria infection (CHMI) with P. falciparum sporozoites in healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against P. falciparum*.

(ii) If subjects from regions where *Babesia microti* transmission is known are included in studies in which this biomarker will be used, consider screening of subjects for *B. microti* infection prior to inclusion in the study as your pan-*Plasmodium* test detects *B. microti as a non-specific entity*.