Statistical analysis plan for

Phase 2 Trial of Safety, Immunogenicity, and Efficacy against *Plasmodium falciparum* Malaria of PfSPZ Vaccine in Children in Mali

1. Populations for Analyses

The following analysis populations will be used when describing subject disposition and performing statistical analyses:

- The **Screened Population** includes all subjects who are screened and provide informed consent, regardless of whether the subject is randomized or treated. This population will be used to fully account for subject disposition.
- The **Intention to Treat (ITT) Population** is a subset of the screened population that is deemed eligible to participate in the study, is randomized to a treatment group, receives *at least one injection* with PfSPZ Vaccine or placebo, and contributes evaluable person-time to the risk set.
- The **Modified Intention to Treat (mITT) Population** includes all subjects who are deemed eligible to participate, are randomized, receive *all three injections* of vaccine or placebo, and contribute evaluable person-time to the risk set, including subjects to whom V2 and/or V3 is administered out of the pre-specified time window or an incomplete injection is given, defined as an estimated injection volume of at least 10% and less than 80%.
- The **According to Protocol (ATP) Population** is a subset of the mITT Population, including subjects who complete the vaccination series per protocol and contribute evaluable person-time to the risk set.

2. Study endpoints

2.1 Safety endpoints

The primary safety endpoint is the proportion of vaccinees experiencing related SAEs from V1 to 26 weeks after V3.

The secondary safety endpoints include the occurrence of unsolicited AEs, laboratory abnormalities, and solicited AEs in the ITT population.

2.2 Efficacy endpoints

The primary efficacy endpoint is the vaccine efficacy against *malaria with symptoms* detected by TBS. TBS are deemed positive if at least one unambiguous asexual blood stage parasite is identified by two independent microscopists after each examining 0.50

µL of blood. For this protocol, there is a primary and a secondary case definition of Pf *malaria with symptoms*.

Primary case definition:
Pf *malaria with symptoms* is defined as a positive thick blood smear at a density of >1000 parasites/uL (P/uL) plus:

- Measured fever ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria:
    - Verbal individual (individual able and willing to answer questions): A verbal individual is considered symptomatic if reporting at the time of evaluation at least two of the following symptoms/symptom groups: headache, chills and/or rigors, malaise and/or fatigue, dizziness and/or light-headedness, myalgias and/or arthralgias; or
    - Non-verbal individual (small child or any individual unable or unwilling to answer questions): A non-verbal individual is considered symptomatic if manifesting at the time of evaluation at least two of the following signs/sign groups: drowsiness, irritability and/or fussiness, inability and/or refusal to eat or drink, prostration; or
- Any individual: Signs of severe malaria.

<u>Secondary case definition:</u>
Pf *malaria with symptoms* is defined as a positive thick blood smear at a density of > 0 P/uL plus:

- Measured axillary temperature ≥ 37.5 degrees Celsius or history of fever (subjective or objective) in the last 24 hours, or,
- Symptoms of malaria as defined in the primary case definition; or
- Meeting criteria for severe malaria

The secondary efficacy endpoint is the vaccine efficacy against Pf malaria.
Pf *malaria* is defined as:

- At least one unambiguous asexual parasite on thick blood smear identified by two independent microscopists after each examining 0.50 µL of blood in a study participant

3. Analysis methods

3.1 Safety Analyses

The primary safety endpoint is the occurrence of an SAE between V1 and 26 weeks after V3 which is at least possibly related to treatment. Serious AEs which meet this definition will be summarized in frequency tables and listings according to system organ class, preferred term, relatedness, and severity. The primary safety analysis will be performed using the ITT population and according to treatment received at V1, regardless of any randomization or allocation errors. All SAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized using frequencies, proportions, and exact 95% CIs for proportions. Rates of SAEs (overall and for any system organ class or preferred term with at least 5 events in either group) will be compared using two-sided 0.05 level Fisher's Exact Tests.

Secondary safety endpoints include the occurrence of unsolicited AEs, laboratory abnormalities, and solicited AEs in the ITT population. Unsolicited AEs documented from V1 to 14 days following V3 will be coded using MedDRA and summarized in frequency tables according to system organ class, preferred term, relatedness, and severity. Summaries will be provided overall and according to the vaccination number immediately prior to AE onset. Rates of unsolicited AEs (overall and for any system organ class or preferred term with at least 5 events in either group) will be compared using two-sided 0.05 level Fisher's Exact Tests. Adverse events leading to premature discontinuation from the study will be listed separately. Clinically relevant laboratory abnormalities recorded in the 14 days after V3 will be summarized in frequency tables by type, grade, and clinical relevance.

3.2 Efficacy Analysis

3.2.1 Primary: VE against Pf *malaria with symptoms*

The primary efficacy analysis will be the modified Intention to Treat (mITT).

The primary efficacy analysis will be based on time to the first Pf malaria with symptoms. Entry into the risk set begins on the latter of a) 14 days after completing the primary vaccination series (V3 + 14 days) or b) 28 days after initiating treatment for a parasitemia event that was detected on or before V3 + 14 days, whichever comes later. Time-to-event is defined as the number of days between entry into the risk set and date of first Pf *malaria with symptoms*; subjects who do not experience the event by 26 weeks after V3 will be censored from the primary analysis or earlier date of last negative TBS result.

The survival patterns will be described by Kaplan-Meier curves for each arm and compared by the logrank test between two arms. The protective efficacy will be assessed from the Cox proportional hazards model with treatment arm as the regressor, and the vaccine efficacy will be estimated as one minus the hazard ratio.

As a secondary efficacy analysis, the cumulative probability of Pf *malaria with symptoms* through 26 weeks post V3 will be presented for each treatment arm and compared across arms based on Kaplan-Meier estimates along with 95% confidence intervals. This estimation accounts for right censoring and the comparison should be equivalent to Fisher's exact test in case of no censoring.

Sensitivity analyses will include an ITT and ATP analysis with entry into the risk set as with the mITT analysis, and ATP analyses with entries into the risk set at time of first vaccination and at V3.

3.2.2 Secondary: VE against Pf malaria

VE against Pf *malaria* will be assessed as for VE against Pf *malaria with symptoms*, using the mITT population and censoring participants who do not experience the endpoint by 6 months after V3.

The cumulative probability of Pf *malaria* through 26 weeks post V3 will be similarly presented as for Pf malaria with symptoms.

3.3 Exploratory Analyses

The trend of the immune response, more specifically the humoral and cellular immune responses, to PfSPZ vaccine will be modelled and compared between the vaccine recipients and the controls. This will be achieved via a generalized linear regression which accounts for the within-subject correlation.

To assess genetic relatedness of the PfSPZ vaccine parasite strain to malaria infection parasites, a genotypic sieve analysis will be performed to analyze the sequences of peripheral blood *Pf* parasites from infected subjects. The sieve analysis targets at differentiating protective efficacy against different genotypes of infection-inducing parasites with genotype defined by the number of mismatches to the PfSPZ footprint.

Recurrent event analysis will be performed to assess vaccine efficacy against all episodes of Pf *malaria with symptoms*, or Pf malaria, rather than against the first episode.

To evaluate the impact of detectable parasitemia prior to vaccination on vaccine efficacy, vaccine efficacy may be additionally evaluated via the above mentioned analyses stratified by baseline status of parasitemia.

3.4 Handling of missing Data

It is anticipated that some event dates required for safety analyses may be incomplete. If the event onset day is unknown, then the date will be imputed as the last date the subject could reliably be assumed to be event free (e.g., the previous study visit where the event was not reported). If the resolution day is incomplete, then the date will be imputed as the date that the event was reliably known to no longer be present.

It is anticipated that some TBS results will be missing. In those instances where one TBS is missed, the result will be imputed to be the same as the result of the next TBS. Up to three such single missing TBS will be allowed in a research subject during the 26 weeks of follow-up without disqualifying the participant from the ATP population (as long as the individual meets other requirements for ATP, such as receiving all three immunizations within the correct time windows). If there are situations where two or more consecutive TBS are missed, the result will again be imputed to be the same as the result of the next TBS, and the research subject will be included in the mITT population (as long as the individual meets other requirements for mITT, such as receiving all three immunizations). If the only TBS missed is the last, there will be no imputation, the missing data point will be ignored, and the individual may be included in the ATP population. If the two or more TBS missed are the last, there will be no imputation, the missing data points will be ignored, and the individual may be included in the mITT population. However, if more than the last two TBS are missed, the individual will not be included in proportional efficacy analyses, although may be included in time-to-event efficacy analyses.

Subjects who discontinue early or are lost to follow-up will be censored from applicable analyses at the last date where the study outcome can reliably be determined (e.g., date of last available TBS specimen without parasitemia).