STATISTICAL ANALYSIS PLAN

SAFETY AND IMMUNOGENICITY EVALUATION OF THE MALARIA VACCINE, RTS,S/AS01, IN HEALTHY THAI ADULTS

(BAKMAL 1605)

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SIGNATURES			
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1 INTRODUCTION

This document details the proposed analysis of primary and secondary objectives for the study "Safety and Immunogenicity Evaluation of the Malaria Vaccine, RTS,S/AS01, in Healthy Thai Adults". The contents of this document are intended to establish rules that will be followed, as closely as possible, when analysing and reporting the trial. Some flexibility will be allowed in handling exploratory analyses.

The statistical analysis plan will be available on request to journals where the manuscripts for this work will be submitted. Any Suggestions/recommendations on the analysis approaches by journal editors or reviewers, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

If during analysis, we note some important issues/approaches not in the Statistical Analysis Plan but worth considering, the deviations will be described and justified in the final report of the trial.

2 BACKGROUND INFORMATION

2.1 OBJECTIVES

2.1.1 PRIMARY OBJECTIVES

- To assess the safety and immunogenicity of the standard formulation of the RTS,S vaccine (RTS,S/AS01E) in Thai adults (Group 3 alone).

- To assess the safety and immunogenicity of the adult formulation of the RTS,S vaccine (RTS,S/AS01B) in Thai adults (Group 1 alone).

- To compare the safety and immunogenicity of a fractional dose RTS,S regime (where the third dose is a 1/5th of the standard dose) using the adult formulation (RTS,S/AS01B) to the safety and immunogenicity of fractional dose RTS,S regimes using either single or double doses of the standard formulation (RTS,S/AS01E), in Thai adults (Group 1 vs Group 2 and Group 1 vs Group 5).

- To compare the safety and immunogenicity of fractional dose regime (where the third dose is a 1/5th of the standard dose) using a single dose of the standard formulation (RTS,S/AS01E) to the safety and immunogenicity of fractional dose RTS,S regime using a double dose of the standard formulation (RTS,S/AS01E), in Thai adults (Group 2 vs Group 5).

- To compare the serologic response to RTS,S/AS01E vaccine when co-administered with DHA-PIP + SLD-PQ with RTS,S/AS01E vaccine alone in adults, when RTS,S/AS01E is given in either a fractional dose regime (where the third dose is a 1/5th of the standard dose) or a full standard dose regime (Group 3 vs. Group 4 and Group 5 vs Group 6).

- To compare the serologic response to RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional two-dose regimen (Month 0, standard dose and Month 2, fractional (1/5th) dose) with RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional three-dose regimen (Month 0 and Month 1, standard doses and Month 2, fractional (1/5th) dose) (Group 6 vs Group 7)

2.1.2 STUDY DESIGN

This is a randomized, open-label, single centre, Phase 2 trial of RTS,S/AS01 in healthy Thai adults. A total of 190 healthy, non-pregnant Thai adults (aged 18 to 55 years, inclusive) will be recruited into the study. They will be randomized into 7 arms as in the table 1 in section 2.4 below.

2.1.3 PATIENT POPULATION

Healthy adults aged 18-55 years.

The inclusion and exclusion are detailed in the protocol section 4.

2.1.4 SAMPLE SIZE

The sample size calculations are based on the objective of comparing the serologic response to RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ with RTS,S/AS01E or RTS,S/AS01B vaccine alone in adults. This is a phase II trial. Sample sizes for phase II trials are usually small, as safety is still of paramount importance. Valid sample sizes for comparing sample sizes between two groups are usually obtained using exact test methods such as the Fisher's exact test power calculations. This is done using simulations as sample size formulas are not well developed. To estimate the sample size we used a four-fold increase in anti –CS antibody titre as an assumed correlate of protection. For the sample size calculation, we assume that the background rate of response in the reference groups will be 5% and the true rate of anti-CS antibody response in the vaccine groups is 80%. We wish to exclude, for the vaccine, a one-tailed lower 95% CI for the reference-vaccine group difference of lower than 30%. The study aims to detect this difference with 90% power (because the detectable difference is big) and testing at 5% significance level. With these assumptions, and using the method of Blackwelder [1] for precision-based sample size calculations, a total of 20 participants per group would be needed for arms 1 and 2 because the anticipated difference is too big. For arms 3 to 7 (Table 3 below), we will need more participants per group because a smaller detectable effect is anticipated. Using the Fisher's exact test power simulations, a difference in serologic response of 30% (say 65% vs 95%) gives approximately 80% power with a sample size of 30 participants in each group testing at 5% significance level. The total sample size for all the 7 arms will be 190 participants.

The size of study groups is relatively small hence it is imperative to minimise potential losses. Whenever possible an attempt will be made to replace study participates should a participant be withdrawn or choose to withdraw. The stata command for obtaining the Fishers exact test power simulations is: power two proportions 0.65 0.95, test(fisher) n1(30) n2(30). This has been performed in Stata 14.

Table 1: Sample size and vaccine vials required for each arm

Group	# participants	Vaccine	Dose M0	Dose M1	Dose M2	Antimalarial	vaccine vials required
1	20	RTS,S/AS01 B	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)		60
2	20	RTS,S /AS01E	Double standard dose (1.0mL)	Double standard dose (1.0mL)	Double Fx dose (0.2mL)		60
3	30	RTS,S/AS01 E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Single standard dose (0.5mL)		90
4	30	RTS,S/AS01	Single	Single	Single	DHA-PIP+PQ	90

Group	# participants	Vaccine	Dose M0	Dose M1	Dose M2	Antimalarial	vaccine vials required
		E	standard dose (0.5mL)	standard dose (0.5mL)	standard dose (0.5mL)		
5	30	RTS,S/AS01 E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)		90
6	30	RTS,S/AS01 E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)	DHA-PIP+PQ	90
7	30	RTS,S/AS01 E	Single standard dose (0.5mL)		Fx dose (0.1mL)	DHA-PIP+PQ	60
Total	190						540

Fx dose= fractional dose

RTS,S/AS01B = adult formulation containing 50µg of RTS,S and 50µg MPL and 50µg QS-21 in the standard dose of 0.5mL.

RTS,S/AS01E = standard formulation containing 25µg of RTS,S and 25µg MPL and 25µg QS-21 in the standard dose of 0.5mL.

DHA-PIP = dihydroartemisinin-piperaquine

PQ = single low dose primaquine

Fx dose = fractional dose i.e. $1/5^{th}$ of standard dose of RTS,S/AS01B or RTS,S/AS01E

2.1.5 RANDOMISATION

Randomization numbers will be generated in blocks, for the 19 intervention arms in a ratio of 2:2:3:3:3:3:3, giving an overall ratio of 20:20:30:30:30:30:30 for groups 1 to 7 respectively as follows:

- RTS,S/AS01B Fractional dose group (Group 1)
- Double RTS,S /AS01E Fractional dose group (Group 2)
- RTS,S/AS01E Standard dose group (Group 3)
- RTS,S/AS01E + DHA-PIP+PQ Standard dose group (Group 4)
- RTS,S/AS01E Fractional dose group (Group 5)
- RTS,S/AS01E + DHA-PIP+PQ Fractional dose group (Group 6)
- RTS,S/AS01E + DHA-PIP+PQ Fractional two-dose group (Group 7)

Study participants were assigned the next available randomization number on the list, and thus were randomly allocated to Group 1, 2, 3, 4, 5, 6 or 7. This is an open-label study and participants and clinical investigators were not blinded to group allocation.

Subjects were vaccinated by intramuscular (IM) needle injection into the deltoid region of the arm. Subjects in Groups 4, 6 and 7 also received anti-malarial medications.

3 PRIMARY AND SECONDARY OUTCOME MEASURES

3.1 Inclusion in Analysis

All patients who received at least one dose of vaccine will be included in the safety analyses. Patients lost to follow-up before the completion of the follow-up period assessments will be censored at the last day seen.

Immunology analyses

Given the incidence of malaria in the study area, it is highly unlikely that any volunteer became infected with any *Plasmodium* species during the trial. However, to exclude the remote possibility that a participant becomes infected we will screen participants for malaria at screening, Month 0, Month 1, Month 2, Month 3, and Month 6. The malaria screen consists of a blood film which is read immediately in the hospital laboratory. The result were available in <30 min. Subjects positive for malaria at screening or Month 0 are excluded from participation in the study and were not enrolled or vaccinated. Subjects positive for malaria at Month 1 or Month 2 were withdrawn from further vaccinations. In the remote scenario that a volunteer becomes parasitaemic during the study results obtained from that point in time onward will be confounded by the immune response to the natural infection. Immunology results obtained during and after an episode of parasitaemia were excluded from the immunology analysis. The results can be included in the safety analysis.

The results will be reported in two ways: a) excluding results for the out-of-window visit as specified in the protocol (analogous to the per protocol approach PP) and b) including the results analogous to an intention to treat ITT approach.

3.2 PRIMARY OUTCOME

The primary outcome measures will be:

- The safety of the investigational vaccine in each group:
    - Occurrence of serious adverse events (SAEs) from the date of the first vaccination to 29 days after the last vaccination, according to the MedRA classification.
    - Occurrence of SAEs during the whole study period, i.e. during a 6 month follow up period from the receipt of first vaccination, according to the MedRA classification.
- For groups 1 to 6, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), one month after the second dose (at Study month 2), one month after the third dose (at Study Month 3) and six months after the first dose (at Study Month 6).
- For group 7, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), two months after the first dose (at Study month 2), one month after the second dose (at Study month 3), and six months after the first dose (at Study Month 6).

4 STATISTICAL METHODS

4.1.1 GENERAL CONSIDERATIONS

4.1.2 DATA CHECKS

Data for each variable including calculated changes from baseline will be summarised using descriptive statistics such as n, mean, median, minimum and maximum, to check for any values that are out of range or implausible. Missing data will be identified and queried when preparing data for analysis.

4.1.3 BASELINE DATA

Patient demographic characteristics (age, gender, weight), and all other baseline information will be summarised for each treatment group.

Numbers (with percentages) for binary and categorical variables. The mean (standard deviation) will be presented for continuous normally distributed data. The median (interquartile or full range) for continuous variables will be presented.

There will be no tests of statistical significance conducted for differences between randomised groups on any baseline variables.

4.1.4 SCREENING AND RECRUITMENT FLOWCHART

A study flowchart showing numbers of participants screened, randomised and those who are available for the final analysis populations will be constructed. Reasons for drop-out or exclusions will be shown at each stage.

4.1.5 ANALYSIS POPULATION

Primary outcome analyses will be carried out on both the according to protocol (PP) and the intention to treat (ITT) population. The PP analyses will be the main strategy for the immunogenicity analyses. The safety outcomes will be ITT. In the intention-to-treat analysis, every participant randomized in the study (who receive the correct or incorrect study agent, one or more doses, and complete or incomplete doses) will be analysed, except if he/she did not receive any dose of the study vaccine or if no post-randomization data was collected for this participant.

The per-protocol analysis will compare participants according to the study agent actually received and will include only those participants who satisfied the inclusion/exclusion criteria, followed the protocol, and received complete, correct doses. The following non-compliant participants will be excluded:

- Participants included without meeting at least one inclusion criterion
- Participants included despite meeting at least one exclusion criterion
- Participants found non-compliant with the blood sampling schedule
- Participants vaccinated with the wrong study agent (non-compliance with the randomization code)
- Participants excluded from the intention-to-treat analysis.

4.1.6 ANALYSES TO ADDRESS PRIMARY AND SECONDARY OBJECTIVES

4.1.7 DESCRIPTIVE ANALYSES

For each study measure descriptive statistics within each randomised group and overall will be

presented for data at each study time point.

Table 1. –Characteristics (M0 Day 0 measurements) of the participants by vaccine group

Characteristic	Group 1 n=X	Group 2 n=X	Group 3 n=x	Group 4 n=x	Group 5 n=x	Group 6 n=x	Group 7 n=x
Gender n (% male)							
Age (years): median (range)							
Weight (kg): median (range)							
QTc int (msec): median (range)							
Fever n (%)							
Hb mean (sd)							
WBC median (range)							
Platelets median (range)							
Creatinine median (range)							
AST median (range)							
ALT median (range)							
Temperature mean (sd)							

4.1.8 Analysis of immunogenicity

The serum anti-CS antibody titre and avidity responses for each treatment arm will be compared to the titre before vaccine administration as outlined in tables 2a, 2b, 3a and 3b below. In addition immunology will be analyzed and compared in terms of the following parameters using appropriate statistics according to the data structure in which data will be presented to the statistician:

- *Plasmodium falciparum*. Circumsporozoite Protein. (NANP)6 Ab.IgG
- *Plasmodium falciparum*. Circumsporozoide Protein. (NANP)6 Ab.IgG Avidity
- *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG
- *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG avidity
- *Plasmodium falciparum*. Circumsporozoite Full length (N+C-Terminal)
- *Plasmodium falciparum*. anti-full length CSP Ab.IgG avidity

The serum anti-CS antibody titre -Proportion in study group with >4-fold rise in titre from baseline to: One month after first dose; One month after second dose; One month after the third dose; Six months after the first dose for groups 1 to 6 AND one month after first dose; two months after first dose; one month after the second dose and Six months after the first dose for group7.

Table 2ai. *Plasmodium falciparum*. Circumsporozoite Protein.(NANP)6 Ab.IgG

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				

Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

Table 2aii. *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

Table 2aiii. *Plasmodium falciparum*. Circumsporozoite Full length (N+C-Terminal)

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

AVIDITY

Avidity -Proportion in study group with >4-fold rise from baseline to: One month after first dose; One month after second dose; One month after the third dose; Six months after the first dose for groups 1 to 6 AND one month after first dose; two months after first dose; one month after the second dose and Six months after the first dose for group7.These will be summarised in tables 2bi to 2bii as follows:

Table 2bi. *Plasmodium falciparum*. Circumsporozoide Protein.(NANP)6 Ab.IgG Avidity

ARM	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7			

Table 2bii. *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG avidity

ARM	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7			

Table 2biii. *P falciparum*. anti-full length CSP Ab.IgG avidity

ARM	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7			

The frequency and the proportions of participants who are seropositive* for antibodies at each timepoint will be summarized in the table below

Table 2ci. Proportion seropositive* for antibodies at each timepoint: *Plasmodium falciparum*. Circumsporozoite Protein.(NANP)6 Ab.IgG

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

** The percentage of subjects seroconverting after each immunization based on a value greater than the mean titer at baseline (before immunization # 1) plus 2 standard deviations for all subjects included in the analysis.*

Table 2cii. Proportion seropositive* for antibodies at each timepoint: *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

** The percentage of subjects seroconverting after each immunization based on a value greater than the mean titer at baseline (before immunization # 1) plus 2 standard deviations for all subjects included in the analysis.*

Table 2ciii. Proportion seropositive* for antibodies at each timepoint: *Plasmodium falciparum*. Circumsporozoite Full length (N+C-Terminal)

ARM	1 month after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	1 month after 3rd dose **n (%)**	6 months after 1st dose **n (%)**
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				

	1 month after 1st dose **n (%)**	2 months after 1st dose **n (%)**	1 month after 2nd dose **n (%)**	6 months after 1st dose **n (%)**
Group 7				

***** *The percentage of subjects seroconverting after each immunization based on a value greater than the mean titer at baseline (before immunization # 1) plus 2 standard deviations for all subjects included in the analysis.*

The serum anti-CS antibody titre

The serum anti-CS antibody titre -The geometric mean-in titre from baseline to: One month after first dose; One month after second dose; One month after the third dose; Six months after the first dose for groups 1 to 6 AND one month after first dose; two months after first dose; one month after the second dose and Six months after the first dose for group 7.

Table 3ai. *Plasmodium falciparum*. Circumsporozoite Protein.(NANP)6 Ab.IgG

ARM	1 month after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose Geometric Mean (95 % CI)	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7				

Table 3aii. *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG

ARM	1 month after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose Geometric Mean (95 % CI)	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7				

Table 3aiii. *Plasmodium falciparum*. Circumsporozoite Full length (N+C-Terminal)

ARM	1 month after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)

Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
	1 month after 1st dose Geometric Mean (95 % CI)	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7				

AVIDITY (Geometric means)

Avidity -The geometric mean in titre from baseline to: One month after first dose; One month after second dose; One month after the third dose; Six months after the first dose for groups 1 to 6 AND one month after first dose; two months after first dose; one month after the second dose and Six months after the first dose for group 7.

Table 3bi. *Plasmodium falciparum*. Circumsporozoide Protein.(NANP)6 Ab.IgG Avidity

ARM	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7			

Table 3bii. *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG avidity

ARM	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7			

Table 3biii. *P falciparum*. anti-full length CSP Ab.IgG avidity

ARM	1 month after 2nd dose Geometric Mean (95 % CI)	1 month after 3rd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 1			
Group 2			
Group 3			
Group 4			
Group 5			
Group 6			
	2 months after 1st dose Geometric Mean (95 % CI)	1 month after 2nd dose Geometric Mean (95 % CI)	6 months after 1st dose Geometric Mean (95 % CI)
Group 7			

5 STATISTICAL ANALYSES OF PRIMARY SAFETY ENDPOINTS

Safety analysis will be performed on patients allocated to receive vaccination (Groups 1, 2, 3, 4, 5 ,6 and 7). Analyses will not be performed for all treatment groups and by treatment group, and no statistical tests will be performed. The percentage of subjects with at least one local AEs with at least one general AE (solicited and unsolicited) and with any AE during the 7-day or total follow-up period up to 29 days after the last vaccine dose and overall will be tabulated with exact 95% CI. The percentage of doses followed by at least one local AE (solicited and unsolicited), by at least one general AE (solicited and unsolicited) and by any AE during the 7-day or the total follow up period up to 29 days after the last vaccine will be tabulated with exact 95% CI. The same computations will be done for Grade 3 AEs, for any AEs considered related to vaccination and for any Grade 3 AEs considered related to vaccination. The percentage of subjects reporting each individual solicited local AE (any grade and Grade 3) and solicited general AE (any grade, Grade 3, any related, Grade 3 related, resulting in medically attended visit) during the 7-day follow-up period (Day 0-6) after each vaccine dose and overall will be tabulated for each group. Similarly, the percentage of doses followed by each individual solicited local and general AE will be tabulated, overall vaccination course, with exact 95% CI. For fever, the number and percentage of subjects reporting fever by half degree (°C) cumulative increments during the first seven days (Day 0-6) after each vaccine dose and overall will be tabulated. Similar tabulations will be performed for any fever with a causal relationship to vaccination and Grade 3 (> 39.5°C) causally related fever. The percentage of subjects reporting unsolicited AEs from the date of the first vaccine dose up to 29 days after the last vaccine dose will be tabulated by group and by MedDRA preferred term with exact 95% CI. Similar tabulation will be done for Grade 3 unsolicited AEs, for any causally related unsolicited AEs and for Grade 3 causally related unsolicited AEs. The percentage of subjects reporting SAEs and pregnancies will be described in detail. The percentage of vaccinated subjects reporting AEs of specific interest (meningitis and pIMDs) will be described in detail.

Thus, in summary, the safety analysis will be as follows:

- Frequency (%) of occurrence of solicited local and general AEs within seven days (day of vaccination and six subsequent days) after each vaccination with exact 95% CI by group and severity.

- Frequency (%) of occurrence of unsolicited AEs from the date of the first vaccination to 29 days after the last vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification with exact 95% CI, by group and severity.

- Frequency (%) of occurrence of SAEs (all, fatal, related to investigational vaccine) within 30 days (day of vaccination and 29 subsequent days) after each vaccination, according to the MedDRA classification with 95% CI.

- Frequency (%) of occurrence of SAEs (all, fatal, related to investigational vaccine) during the whole study period according to the MedDRA classification with exact 95% CI.

- Frequency (%) of occurrence of AEs and SAEs leading to withdrawal from further vaccination from Dose 1 (Day 0) up to study conclusion (Day 180), according to the MedDRA classification, for each vaccinated subject exact 95% CI.

- Frequency (%) of occurrence of pIMDs from Dose 1 (Day 0) up to study conclusion (Day 180), according to MedDRA classification, for each vaccinated subject with exact 95% CI.

- Frequency (%) of occurrence of meningitis from Dose 1 (Day 0) up to study conclusion (Day 180), according to the MedDRA classification, for each vaccinated subject exact 95% CI.

These will be summarised in table 4 below:

Table 4 Safety analysis

Safety parameter	Group1 n=X	Group2 n=X	Group3 n=x	Group4 n=x	Group5 n=x	Group 6 n=x	Group7 n=x
Solicited local and general AEs within seven days n(%) (95% CI)							
Unsolicited AEs: date of 1st vaccine to 29 days after last vaccine n(%), (95% CI)							
SAEs within 30 days after each vaccine n (%), (95% CI)							
SAEs for whole period n (%) (95% CI)							
AEs+SAEs leading to withdrawal from further vaccination from Dose 1 to study conclusion n(%) (95% CI)							
pIMDs from Dose 1 to study conclusion n(%), (95% CI)							
Meningitis from Dose 1 to study conclusion n(%), (95% CI)							

Biochemistry and haematological safety assessment.

Biochemistry (ALT, AST and creatinine) and haematological (haemoglobin, WBC, and platelets) laboratory values will be presented according to toxicity grading scales and tabulated by group. The normal ranges and toxicity grading for laboratory safety parameters those will be used in this study are in Appendix section (Table A1):

The following definitions will be used for clinical adverse events in this report: The intensity should be assigned to one of the following categories:

1 (mild) = An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

2 (moderate) = An AE which is sufficiently discomforting to interfere with normal everyday activities.

3 (severe) = An AE which prevents normal, everyday activities. In adults, such an AE would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3.

Laboratory Profiles for those participants who had at least grade one toxicity during the trial

Table 5a: Hb profile in participants with abnormal Hb values by group (n=X)

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table 5b: WBC profile in participants with abnormal WBC values by group (n=X)

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table 5c: Platelets profile in participants with abnormal platelet values by group (n=X)

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table 5d: Creatine profile in participants with abnormal Creatinine values by group (n=X)

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table 5e: AST profile in participants with abnormal AST values by group (n=X)___

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table 5f: ALT profile in participants with abnormal ALT values by group (n=X)

Anonymized ID	gender	group	screening	M0 D0	M0 D7	M1 D7	M2 D7	M3

Colour Code Key: grade1=green; grade 2=blue; grade3=Pink; grade 4=red; CL=clotted sample; HML=Haemolysed sample; WD=withdrew

Table A1. The normal ranges and toxicity grading for laboratory safety parameters

Adverse event	Intensity grade	Intensity*
Haemoglobin (males)	Normal range	12.5 - 17.0 g/dl
	1	< 12.5 but ≥ 11.0 g/dl
	2	< 11.0 but ≥ 10.0 g/dl
	3	< 10.0 g/dl
Haemoglobin (females)	Normal range	11.5 - 15.0 g/dl
	1	< 11.5 but ≥ 10.5 g/dl
	2	< 10.5 but ≥ 9.5 g/dl
	3	< 9.5 g/dl
Increase in leukocytes (WBC)	Normal range	3200 - 10799 cells/mm^3
	1	10800 - 15000 cells/mm^3
	2	15001 - 20000 cells/mm^3
	3	> 20001 cells/mm^3
Decrease in leukocytes (WBC)	Normal range	3200 - 10800 cells/mm^3
	1	2500 - 3199 cells/mm^3
	2	1500 - 2499 cells/mm^3
	3	< 1500 cells/mm^3
Decrease in platelets	Normal	140000 - 400000 cells/mm^3
	1	125000 - 139000 cells/mm^3
	2	100000 - 124000 cells/mm^3
	3	< 100000 cells/mm^3
Alanine Aminotransferase	Normal range	Below ULN (60 U/l for males; 40 U/l for females
	1	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 5 x ULN
Aspartate Aminotransferase	Normal range	Below ULN (40 U/l for males; 35 U/l for females
		1.1 - 2.5 x ULN
		2.6 – 5 x ULN
		> 5 x ULN
Creatinine (males)	Normal range	0.5 - 1.39 mg/dl
	1	1.4 - 1.79 mg/dl
	2	1.8 - 2.0 mg/dl
	3	> 2.0 mg/dl
Creatinine (females)	Normal range	0.5 - 1.29 mg/dl
	1	1.3 – 1.69 mg/dl
	2	1.7 – 1.9 mg/dl
	3	>1.9 mg/dl

ULN: upper limit of normal range

Derived from Study protocol version 5.0 dated 2 Nov 2017 (page38)