Statistical Analysis Plan Amendment 3

Study ID: 215301

Official Title of Study: A Phase 1/2a, open-label, randomized, controlled, multicountry, dose-escalation study to assess the safety and immunogenicity of AS37 in combination with the Hepatitis B surface antigen (HBsAg), according to a 0-1-month schedule, in healthy, HBs-naïve, adults aged 18-45 years

NCT number: NCT05561673

Date of Document: 28-Jun-2024

Note: The typo appears in the following sections of the Statistical Analysis Plan document - 1.1 (Objectives) and Table 1 (Study Interventions). The phrase currently reads “absolute values and changes”, but it should be “absolute values of changes”.

Information Type:	Statistical Analysis Plan (SAP) for Main and Final analysis

TITLE PAGE

Protocol Title:	A Phase 1/2a, open-label, randomized, controlled, multi-country, dose-escalation study to assess the safety and immunogenicity of AS37 in combination with the Hepatitis B surface antigen (HBsAg), according to a 0-1-month schedule, in healthy, HBs-naïve, adults aged 18-45 years
Study Number:	215301 (EARLY-CLINRES-017)
Compound Number:	GSK2231392A
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Sponsor Name: GlaxoSmithKline Biologicals SA (GSK)

Regulatory Agency Identifier Number

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VERSION HISTORY

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	12 Sep 2022	Amendment 1 (10 August 2022)	Not Applicable	Original version
SAP Amendment 1	22 May 2023	Amendment 2 (01-Mar-2023)	CCI [redacted]	
SAP Amendment 2	11 Dec 2023	Amendment 3 (17-Nov-2023)	Amendment of Section 5 to address changed target sample size and minor adjustments in Sections 3 and 4	Protocol Amendment 3 change in target sample size
SAP Amendment 3	28 Jun 2024	Amendment 3 (17-Nov-2023)	Amendment on Section 4.2.1 to reference to the newly added Appendix 6.2. Addition of Appendix 6.2.	Inclusion of Electronic Clinical Outcome Assessment (eCOA) Compliance analysis description

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to describe the planned main and final analyses on primary and secondary endpoints for study EARLY-CLINRES-017 (ECR-017 - 215301). CCI [redacted]

There is a separate SAP for the analyses made for the internal Safety Review Team (SRT) that will oversee this study. An additional SAP might be developed later for other tertiary endpoints analyses.

1.1. Objectives, Estimands and Endpoints

The table below reports the endpoints in scope of this SAP. CCI [redacted]

Objectives	Endpoints and Estimands
Primary	
To evaluate the reactogenicity and safety in all study groups.	• Percentage of participants with solicited administration site and systemic AEs within 14 days (Day 1 till Day 14) after Dose 1 and Dose 2. • Duration of solicited AEs (administration site and systemic) after Dose 1 and Dose 2. • Percentage of participants with any unsolicited AEs within 31 days (Day 1 till Day 31) after Dose 1 and Dose 2. • Percentage of participants with SAEs, MAEs and AEs leading to study withdrawal throughout the entire study period. • Percentage of participants with pIMDs throughout the entire study period. • The absolute values and changes in hematology and biochemistry parameters post-Dose 1 (Day 8 and Day 31) and post-Dose 2 (Day 38 and Day 61) from baseline (pre-vaccination, Day 1). • Percentage of participants with abnormal laboratory parameter values at pre-vaccination (Day 1), post-Dose 1 (Day 8 and Day 31) and post-Dose 2 (Day 38 and Day 61).
Secondary	
To evaluate the humoral immune response in all study groups.	• GMC of Anti-HBs Ab concentrations at Day 1, Day 31, Day 61 and Day 361. • Anti-HBs seroconversion and seroprotection rates at Day 31, Day 61 and Day 361.
CCI [redacted]	

AE = adverse event; **SAE** = serious adverse event; **MAE** = medically attended event; **pIMD** = potential immune-mediated disease; **GMC** = geometric mean concentration; **HBs** = Hepatitis B surface antigen; **Ab** = antibody; CCI [redacted].

Primary estimand

The primary clinical question of interest is to assess the reactogenicity and safety in all study groups among participants who received at least one dose of the study interventions (i.e., the Exposed Set [ES]).

The estimand is described by the following attributes:

- Population: ES.
- Treatment condition: 5 study interventions as described in Table 1.

Table 1 Study interventions

	Study intervention 1	Study intervention 2	Study intervention 3	Study intervention 4	Study intervention 5
Study intervention name:	*Engerix-B*	HBsAg/AS03A	*Fendrix*	HBsAg/AS37B*	HBsAg/AS37A**
Group label	HBs-Alum	HBs-AS03	HBs-AS04	HBs-AS37_50	HBs-AS37_100

*AS37B refers to aluminum hydroxide + 50 µg CCI

**AS37A refers to aluminum hydroxide + 100 µg CCI

- Variable / endpoint:
  - Occurrence of events reported within protocol-defined safety follow-up period.
  - Absolute values post-Dose 1 and 2 and changes from baseline in hematology and biochemistry parameters
- Summary measure:
  - Percentage of participants reporting events within protocol-defined safety follow-up period.
    - For solicited events, the participants not completing the diary are not considered in the denominator (see Section 6.3.2 for details).
    - For unsolicited events, participants not reporting events are considered to be participants without event (see Section 6.3.2 for details).
  - Summary statistics of absolute values post-Dose 1 and 2 and changes from baseline in hematology and biochemistry parameters as well as percentage of participants with abnormal laboratory parameter values, based on on the Food and Drug Administration (FDA) grading as presented in Table 22 of the study protocol.

Secondary estimand

The secondary clinical question of interest is to assess the humoral immune response in all eligible participants who received all doses as per protocol, had immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination (i.e., the Per Protocol Set [PPS]).

The estimand is described by the following attributes:

- Population: PPS. If in any study group, the percentage of vaccinated participants with serological results excluded from the PPS is more than 10%, a second analysis based on the ES will be performed to complement the PPS analysis.
- Treatment condition: 5 treatment groups as described in Table 1.
- Variable / endpoint:
  - Anti-hepatitis B surface antigen (HBs) antibody (Ab) concentrations at Day 1, Day 31, Day 61 and Day 361.
  - Anti-HBs seroconversion and seroprotection status at Day 31, Day 61 and Day 361.
- Summary measure:
  - Geometric Mean Concentrations (GMC) of Anti-HBs Ab.
  - Percentage of participants above seroconversion and seroprotection thresholds.
    - Participants not having results for a specific visit (but being part of the PPS) will not be considered in the denominator, no imputation will be done (see Section 6.3.2 for details).

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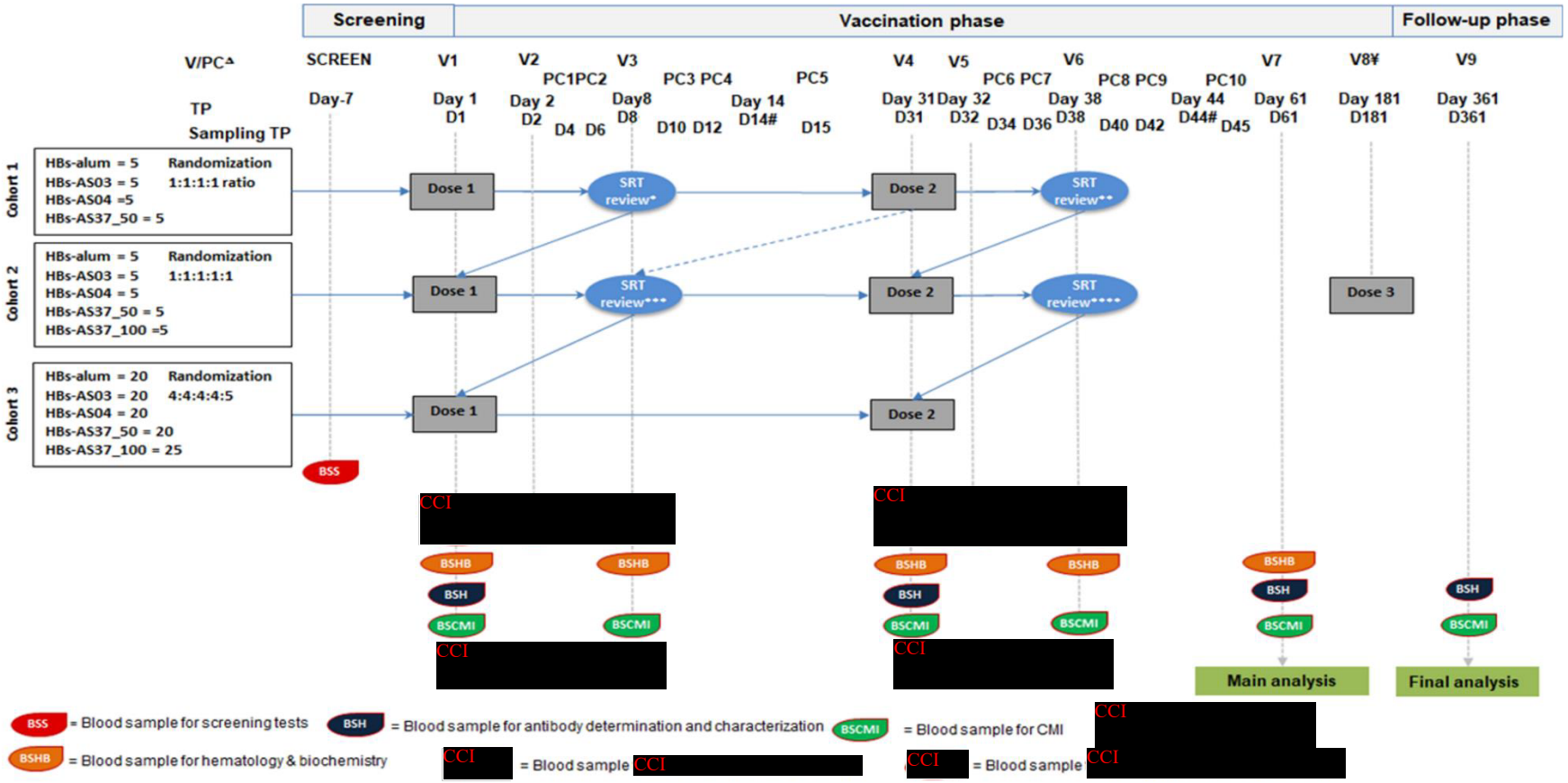
1.2. Study Design

Figure 1 Study design overview



V = visit; **PC** = phone contact; **D** = Day; **N** = number of participants; **SRT** = safety review team; **TP** = timepoint; CCI

¥Only for participants in the HBs-alum group

∆Phone contact at Day 4, Day 6, Day 10, Day 12, Day 15, Day 34, Day 36, Day 40, Day 42, and Day 45

*First safety review after the first 5 participants in the HBs-AS37_50 group have completed 7 days safety follow-up post-Dose 1, before administering the second dose to these participants, and before enrolment of the remaining participants in this group in the next cohorts, and the first 5 participants in the HBs-AS37_100 group in the second cohort. The enrolment in the second cohort will start after the second dose is administered to all 20 participants in the first cohort.

**Second safety review after the first 5 participants in the HBs-AS37_50 group have completed 7 days safety follow-up post-Dose 2, before administering the second dose to the remaining participants in this group in the next cohorts.

***Third safety review after the first 5 participants of the HBs-AS37_100 group have completed 7 days safety follow-up post-Dose 1, before administering the second dose to these participants, and before enrolment of the remaining participants in this group in the third cohort. The enrolment in the third cohort will start after the third SRT review.

****Fourth safety review after the first 5 participants in the HBs-AS37_100 group have completed 7 days safety follow-up post-Dose 2, before administering the second dose to the remaining participants in this group in the third cohort.

#Timepoints for safety data cut-off.

CCI [redacted]

Note: Cohort 3 will enroll a minimum of 70 (and maximum of 105) participants distributed between the groups as follows: a minimum of 23 (and maximum of 30) participants in HBs- Alum, HBs-AS03, HBs-AS04, HBs-AS37_50, HBs AS37_100

- **Type of study**: Self-contained.
- **Experimental design**: Phase 1/2a, dose-escalation, open-label, multi-country study with 5 parallel groups.
- **Duration of the study**: ~ 361 days for all participants.
- **Primary completion date**: Day 361.
- **Control**: Active comparator (*Engerix-B). Other active comparators:* HBsAg/AS03$_A$, *Fendrix*
- **Blinding**: Open-label.
- **Data collection**: Standardized electronic Case Report Form (eCRF). Solicited events will be collected using a Participant Diary (eDiary).
- **Safety monitoring**: Performed by an internal SRT.
- **Study groups**: Refer to Figure 1 and Table 2 for an overview of the study groups.

1.2.1. Staggered administration of study intervention

There will be 3 different cohorts recruited into this study, which will be enrolled in a staggered way. Cohort 1 will enroll 20 participants distributed between the groups as follows: 5 in HBs-Alum, 5 in HBs-AS03, 5 in HBs-AS04, and 5 in HBs-AS37_50. Cohort 2 will enroll 25 participants distributed between the groups as follows: 5 in HBs-Alum, 5 in HBs-AS03, 5 in HBs-AS04, 5 in HBs-AS37_50, and 5 in HBs-AS37_100. Cohort 3 will enroll a minimum of 70 (and maximum of 105) participants distributed among the groups to reach a total sample size across the three cohorts as follows: a minimum of 23 (and maximum of 30) participants in HBs-Alum, HBs-AS03, HBs-AS04, HBs-AS37_50, and HBs-AS37_100. Sample sizes per group are presented in terms of minimums and maximums to allow early stop of enrollment due to reasons of feasibility linked to recruitment of the study population. The choice of the minimum sample size for each group is guided by statistical considerations on the precision of the safety evaluation and on the power for the immunogenicity comparison. For more details, refer to Section 5.

There will be a screening phase whereby participants will be screened for eligibility before recruitment. The study is planned to be conducted at sites in multiple countries.

Table 2 Study groups, intervention and blinding

Study groups	Number of participants	Age (Min-Max)	Study interventions	Timing of intervention		
				Visit 1	Visit 4	Visit 8
HBs-alum	23-30	18-45 years	*Engerix-B*	X	X	X
HBs-AS03	23-30		HBsAg/AS03$_A$	X	X	
HBs-AS04	23-30		*Fendrix*	X	X	
HBs-AS37_50	23-30		HBsAg/AS37B*	X	X	
HBs-AS37_100	23-30		HBsAg/AS37A**	X	X	

*AS37B refers to aluminum hydroxide + 50 µg CCI [redacted]
**AS37A refers to aluminum hydroxide + 100 µg CCI [redacted]

The study will be conducted following a staggered design, and the initiation of the HBs-AS37_100 group will be conditional to whether any holding rule is met in the HBs-AS37_50 group.

Study holding rules are defined in Table 3. Holding rules 1a-d will be assessed by the investigator on a continuous basis and meeting any of these holding rules will trigger a hold of vaccination irrespective of number of participants enrolled and/or timing of the event relative to vaccination. Holding rules 2a-d will be assessed by the SRT during the scheduled meetings for evaluation of safety data.

Table 3 Study holding rules

Holding rule	Event	Number/percentage of participants per group
1a	Death or any life-threatening SAE regardless of causality	≥1
1b	Any serious adverse event (SAE) considered at least possibly related to the study intervention as per Investigator or Sponsor assessment	≥1
1c	Any withdrawal from the study (by investigator or participant request) following a Grade 3 AE	≥1
1d	Any administration site or systemic solicited AE leading to hospitalization or necrosis at the injection site, each with an event onset within the 7-day (Day 1-7) post-study intervention period	≥1
2a	Any Grade 3 solicited administration site AE (lasting 48h or more as Grade 3) in an investigational group, with an event onset within the 7-day (Day 1-7) post-study intervention period	20%
2b	Any Grade 3 solicited systemic AE (lasting 48h or more as Grade 3) in an investigational group, with an event onset within the 7-day (Day 1-7) post-intervention period	20%
2c	Any Grade 3 unsolicited AE in an investigational group, that can be reasonably attributed to the study intervention, with an event onset within the 7-day (Day 1-7) post-study intervention period or Any Grade 3 or above abnormality in pre-specified hematological or biochemical laboratory parameters* in an investigational group at Day 8 post-study intervention	20%
2d	Any Grade 3 non-serious AE considered as, at least, possibly related to the study intervention as per Investigator or Sponsor assessment, independent of within or not within the same system-organ-class	≥2

AE = adverse event; **SAE** = serious adverse event

1.2.2. Sequence of analyses

There will be no interim analyses.

A main analysis is planned on data collected post-Dose 2 (Day 61 – Visit 7) and a final analysis including data up to study end (Day 361 - Visit 9). However, there will be interim safety monitoring reviews as described in Section 1.2.1. A separate SAP is developed to describe the analyses for the interim safety monitoring reviews.

1.2.2.1. Main analysis

The main analysis will be performed on safety and reactogenicity data (primary endpoints) and immunogenicity data (secondary endpoints) when all data post-Dose 2 (Day 61 – Visit 7) are cleaned and available. CCI [redacted].

1.2.2.2. Final analysis

The final analysis on primary, secondary and CCI [redacted] (safety, reactogenicity and immunogenicity data) will be performed when all data up to Day 361 (Visit 9) are cleaned and available.

An integrated CSR containing all data will be written and made available to the investigators.

2. STATISTICAL HYPOTHESES

No confirmatory statistical hypotheses are to be tested. All analyses are descriptive.

2.1. Multiplicity Adjustment

In general, results will not be adjusted for multiple comparisons.

3. ANALYSIS SETS

Analysis Set	Description	Analyses Evaluated
Screened	All participants who were screened for eligibility.	Study Population
Enrolled	All participants who entered the study (who were randomized or received study intervention or underwent a post-screening procedure). NOTE: Screening failures (who never passed screening even if rescreened) and participants screened but never enrolled into the study (i.e., met eligibility but not needed) are excluded from the Enrolled Set as they did not enter the study.	Study Population
Exposed (ES)	All participants who received at least 1 dose of the study intervention. Analysis per group using the Enrolled Set is based on the administered intervention.	Safety
Per-Protocol (PPS)	All eligible participants who received all doses as per protocol, had immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination.	Immunogenicity
CCI [redacted]		

3.1. Criteria for eliminating data from Analysis Sets

Elimination codes will be used to identify participants to be eliminated from the analyses. Details are provided below for each analysis set. See Table 4 for a list and description of all elimination codes.

3.1.1. Elimination from Enrolled set

Code 800 (fraudulent data), and code 900 (invalid informed consent) will be used to identify participants eliminated from the Enrolled Set.

3.1.2. Elimination from Exposed Set (ES)

Code 1030 (study intervention not administered at all, but participant number allocated), code 800 (fraudulent data) and code 900 (invalid informed consent or post-hoc refusal for data use) will be used to identify participants eliminated from the ES.

3.1.3. Elimination from Per-protocol Set (PPS)

A participant will be excluded from the per-protocol set (PPS) for analysis of humoral immunogenicity in the following manner:

- For codes 800, 900, 1030, 1050 and 2010: participants will be eliminated for all visits.
- For codes 1040, 1070, 1080, 1090, 2040, 2050 and 2080: participants will be eliminated from the specific visit at which the condition is met and onwards.
- For codes 2090, 2100, 2110, 2120: participants will be eliminated at the specific visit at which the condition is met.

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Table 4 List of elimination codes

Code	Condition under which the code is used	Visit when it is applicable	Applicable for analysis set/endpoint
800	Fraudulent data	All	Enrolled set, ES and PPS, CCI
900	Invalid informed consent	All	Enrolled set, ES and PPS, CCI
1030	Study intervention not administered at all, but participant number allocated	All	ES, PPS, CCI
1040	Administration of concomitant vaccine(s) forbidden in the protocol: • Use of any investigational or non-registered vaccine other than the study interventions during the period beginning 30 days before the first dose of study intervention, or their planned use during the study period. • Planned administration/administration of a vaccine* not foreseen by the Protocol in the period starting 30 days* before each dose and ending 30 days* after each dose of study intervention administration**. With the exception of influenza vaccine (pandemic or seasonal). **In case an emergency mass vaccination for an unforeseen public health threat (e.g., a pandemic) is recommended and/or organized by public health authorities outside the routine immunization program, the time period described above can be reduced if, necessary for that vaccine, provided it is used according to the local governmental recommendations and that the Sponsor is notified accordingly.	From the specific visit the condition is met	ES, PPS, CCI
1050	Randomization failure: Participant not treated according to regimen assigned by randomization	From the specific visit (1, 4 or 8) the condition is met	PPS and CCI
1070	Vaccine administration not according to protocol Incomplete vaccination course Participant was vaccinated with the correct vaccine but containing a lower volume Wrong replacement or study vaccine administered (not compatible with the vaccine regimen associated to the treatment number) Route of the study vaccine is not intramuscular Wrong reconstitution of administered vaccine	From the specific visit (1, 4 or 8) the condition is met and from the specific visit (1 or 4) the condition is met but only up until the visit before the next dose for CCI	PPS, CCI
1080	Vaccine temperature deviation Vaccine administered despite a Good Manufacturing Practices (GMP) no-go temperature deviation	From the specific visit (1, 4 or 8) the condition is met for PPS and from the specific visit (1 or 4) the condition is met but only up until the visit before the next dose for CCI	PPS, CCI
1090	Expired vaccine administered	From the specific visit (1, 4 or 8) the condition is met for PPS and from the	PPS, CCI

Code	Condition under which the code is used	Visit when it is applicable	Applicable for analysis set/endpoint
		specific visit (1 or 4) the condition is met but only up until the visit before the next dose for CCI	
2010	Protocol violation linked to inclusion/exclusion criteria All inclusion/exclusion criteria defined in the protocol to be checked except for CCI	All	PPS
5010	Protocol violation linked to inclusion/exclusion criteria All inclusion/exclusion criteria defined in the protocol to be checked including for CCI	All	CCI
2040	Administration of any medication forbidden by the protocol • Use of any investigational or non-registered product (drug, vaccine or medical device) other than the study intervention during the period beginning 30 days before the first dose, or use during the study period. • Administration or planned administration of long-acting immune-modifying drugs at any time during the study period (e.g., infliximab). • Administration of immunoglobulins and/or any blood products or plasma derivatives during the period starting 3 months before the first dose of study intervention or planned administration during the study period. • Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 3 months prior to the first study intervention dose. For corticosteroids, this will mean prednisone equivalent ≥ 20 mg/day, or equivalent. Inhaled, intra-articular and topical steroids are allowed.	From the specific visit the condition is met.	PPS
2050	Intercurrent medical condition: Participants may be eliminated from the PPS for immunogenicity if, during the study, they incur an intercurrent medical condition that could alter their immune response (i.e., varicella, HIV, lymphoma) or are confirmed to have an alteration of their initial immune status.	From the specific visit the condition is met.	PPS
2080	Participants did not comply with vaccination schedule: • Number of days between Visit 1 and Visit 4 is outside [21-42 days] for all study groups • Number of days between Visit 4 and Visit 8 is outside [136-164 days], for HBs-alum group	From the specific visit the condition is met.	PPS
2090	Participants did not comply with blood sample schedule (any of the below options): • Number of days between Visit 1 and Visit 4 is outside [21-42] days • Number of days between Visit 4 and Visit 7 is outside [21-42 days] • Number of days between Visit 7 and Visit 9 is outside [286-314 days]	At the specific visit (4 or 7 or 9) the condition is met	PPS
2100	Serological antibody results not available.	At the specific visit (1 or 4 or 7 or 9) the condition is met	PPS

Code	Condition under which the code is used	Visit when it is applicable	Applicable for analysis set/endpoint
2110	Blood sample for serological antibodies available but not yet tested (main analysis)	At the specific visit (1, or 4, or 7, or 9) the condition is met	PPS
2120	Issues in the lab results that are not related to handling of lab samples (Eg: Hemolysis, calibration of instruments) Unreliable released data as a result of confirmed sample mismatch or confirmed inappropriate sample handling at lab	At the specific visit the condition is met	PPS
CCI [redacted]			

4. STATISTICAL ANALYSES

4.1. General Considerations

4.1.1. General Methodology

Enrolled participants who prematurely withdraw from study will not be replaced.

In the case of the wrong stratification assigned at the time of randomization, the analyses will be performed based on the actual stratum per data collected in the eCRF.

Confidence intervals (CIs) will use 95% confidence levels, unless otherwise specified.

In general, CIs for proportions will be calculated CCI [redacted]. All continuous data will be summarized using descriptive statistics (n, mean, standard deviation [SD], median, minimum and maximum) and categorical data will be summarized using the number and percentage of participants in each category.

GMCs of antibody concentrations and related CIs will be obtained by computing average and CI using the t-distribution on log10-transformed data, and subsequently back-transforming these to the original scale.

4.1.2. Definitions

- For all endpoints, the baseline value will be the latest pre-dose assessment with a non-missing value. If sample collection time is not recorded, Day 1 assessments are assumed to be taken prior to first dose and used as baseline.
- A seronegative participant will be defined as a participant whose antibody concentration is below the cut-off value of the assay (CLIA anti-HBs titer < 6.2 mIU/mL).
- A seropositive participant is a participant whose antibody concentration is greater than or equal to the cut-off value of the assay (CLIA anti-HBs titer ≥ 6.2 mIU/mL).
- Seroprotection threshold is CLIA anti-HBs titer > 10 mIU/mL.
- Seroconversion threshold is CLIA anti-HBs titer > 6.2 mIU/mL.
- CCI [redacted]

4.2. Primary Endpoints Analyses

4.2.1. Analysis of safety planned in the protocol

The primary endpoints will be based on the reactogenicity and safety objectives and will be performed on the ES. Below the follow-up period for solicited AEs is intended for Dose 1 and Dose 2 only and not Dose 3.

- The percentage of participants reporting at least one solicited adverse event (AE), at least one solicited administration site AE, and at least one solicited systemic AE during the 14-day follow-up period (i.e., the day of vaccination and 13 subsequent days) will be tabulated for each group after each dose and overall. For solicited AEs, percentages will be computed based on the total number of solicited symptoms screens completed.
- The number and percentage of participants reporting each individual solicited AE during the 14-day follow up period post-vaccination (i.e., the day of vaccination and 13 subsequent days) will be tabulated with exact 95% CI and descriptive statistics for each group, after each dose and overall.
- The number and percentage of participants reporting each individual solicited administration site AE (any grade, Grade 3 and resulting in medically attended visit) and solicited systemic AE (any grade, Grade 3 and resulting in medically attended visit) during the 14-day follow-up period (i.e. the day of vaccination and 13 subsequent days) will be tabulated for each group, after each dose and overall.

- The number and percentage of participants with each solicited administration site event and separately, solicited systemic event (any grade and grade 3) within the 14-day follow up period will be represented graphically with a bar plot for each group and after each dose.
- The duration of each individual solicited AE during the 14-day follow-up period will be tabulated using descriptive statistics (mean, minimum, Q1, median, Q3, maximum). The same computations will be done for Grade 3 solicited events. This analysis will be done for each group after each dose.
- The number and percentage of participants reporting each individual unsolicited AE category during the 31-day follow up period post-vaccination (i.e., the day of vaccination and 30 subsequent days) will be tabulated with exact 95% CI by event category (e.g., all, grade 3, related, grade 3 related, serious adverse events [SAEs], fatal etc.) for each group, after each dose and overall. Percentages will be computed based on the total number of exposed participants. Participants not reporting an AE will be considered as not having experienced any AE.
- The number and percentage of participants with any unsolicited AEs during the 31-day follow-up period (i.e. the day of vaccination and 30 subsequent days) will be tabulated with exact 95% CI by study group and categorized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT) (i.e. the table will be grouped by SOC and then preferred terms will be included within each group). Similar tabulation will be done for Grade 3 unsolicited AEs, for any causally related unsolicited AEs, for Grade 3 causally related unsolicited AEs and for unsolicited AEs resulting in a medically attended visit and at least one solicited or unsolicited adverse event excluding SAE. The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and symptoms will be coded according to the MedDRA Dictionary for Adverse Reaction Terminology. Every verbatim term will be matched with the appropriate preferred term. This will be done by group, after each dose and overall. The summary tables without SOC and PT information will be also produced for the follow-up period lasting until study end.
- The number and percentage of participants reporting each individual unsolicited AE during the 31-day follow-up period (i.e. the day of vaccination and 30 subsequent days) will be tabulated and ranked by MedDRA PT with the most common appearing first. This analysis will be done for each group after each dose and overall.
- The number and percentage of participants with at least one solicited or unsolicited medically attended event (MAE) reported any time between the day of vaccination (Day 1) and the end of the study period post vaccination (up to Day 61 for main analysis, up to Day 361 for final analysis) will be classified by MedDRA SOC and PT and tabulated with exact 95% CI. This analysis will be done by group.
- The number and percentage of participants with at least one serious adverse event (SAE) reported any time between the day of vaccination (Day 1) and the end of the study period post vaccination (up to Day 61 for main analysis, up to Day 361 for final analysis) will be classified by MedDRA SOC and PT and tabulated with exact 95% CI. This analysis will be done by group. The same tabulation will be presented for causally related SAEs, fatal SAEs and causally related fatal SAEs.

- The number and percentage of participants with at least one potential immune-mediated disease (pIMD) (selection defined by GSK list of terms AND/OR investigator tick box) reported any time between the day of vaccination (Day 1) and the end of the study period post-vaccination (up to Day 61 for main analysis, up to Day 361 for final analysis) will be classified by MedDRA SOC and PT and tabulated with exact 95% CI. This analysis will be done by group. The same tabulation will be presented for causally related pIMDs.
- The number and percentage of participants reporting each individual pIMD, SAE, or MAE that occurred in the study up to study end/database freeze (DBF) prior to the main analysis will be tabulated and categorized by MedDRA SOC and PT with exact 95% CIs, after each dose and overall. This analysis will be done by group. Note: different groups and doses will have different amounts of follow up time.
- All SAEs/MAEs/pIMD will be described in detail in a tabular listing.
- All AEs/SAEs leading to study/intervention discontinuation from dose 1 up to study end/DBF for main analysis will be tabulated and described in detail in a tabular listing.
- All participants taking concurrent medication/vaccines will be described in a detailed listing.
- For each group and for each hematology and biochemistry parameter, as reported in Table 22 of the study protocol:
  - Comparison of changes since baseline i.e., summary of absolute and relative differences for each parameter by timepoint and by group will be tabulated with descriptive statistics.
  - The number and percentage of participants having hematology and biochemistry results below or above the central laboratory defined normal ranges will be tabulated by timepoint [pre-vaccination (Day1), post-dose 1 (Day 8 and Day 31), post-dose 2 (Day 38 and Day 61)]. This will be done for each group and overall.
  - The summary of grading post-study intervention administration will be tabulated vs. baseline. Grades will be based on the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (see Section 10.2.1 of the protocol). The laboratory parameters not included in the FDA Toxicity Grading Scale will not be graded.
- Compliance in completing solicited events information will be tabulated after each dose and overall. The number of completed eDiary days in the solicited period for a participant will be summarized by study group using frequency table. Refer to Section 6.2 for more information.
- Pregnancy and pregnancy outcomes will be listed (if applicable).

- The percentage of participants using concomitant medication (any medication, any antipyretic, and any antipyretic taken prophylactically, respectively) during the 14- day follow-up period (i.e., the day of vaccination and 13 subsequent days) and during the 31-day follow-up period (i.e., the day of vaccination and 30 subsequent days) will be summarized by group after each dose and overall.
- Number and percentage of participants meeting each holding rule will be tabulated with exact 95% CI. This analysis will be done by group and for each dose. Furthermore, it will be done separately by cohort 1, cohort 2 as well as the two together (cohort 1 and cohort 2 combined).

4.2.2. Additional considerations

Solicited AEs will be coded by MedDRA (using the latest version) as per the codes in Table 5:

Table 5 Solicited AEs MedDRA codes

Solicited symptom	Lower level term code	Lower level term
Arthralgia	10003239	Arthralgia
Chills	10008531	Chills
Diarrhea	10012727	Diarrhea
Erythema	10074796	Administration site erythema
Fatigue	10016256	Fatigue
Fever	10016558	Fever
Headache	10019211	Headache
Loss of appetite	10003028	Appetite lost
Malaise	10025482	Malaise
Myalgia	10028411	Myalgia
Nausea	10028813	Nausea
Pain	10058049	Administration site pain
Swelling	10075107	Administration site swelling
Vomiting	10047700	Vomiting

4.3. Secondary Endpoints Analyses

The analysis of the secondary objective on humoral immune response (anti-HBs antibody concentrations) will be performed on the PPS. The main analysis will be performed on data up to post-Dose 2 (Day 61 – Visit 7) from all cohorts (1-3). The final analysis will be performed on data up to Day 361 (Visit 9). No alpha adjustment is planned for the main analysis.

4.3.1. Within group evaluation

4.3.1.1. Analytical Approach

The within-group evaluation will derive unadjusted GMCs based on the anti-log of the mean of anti-HBs log10-concentrations.

Note: Unless otherwise specified, results below the cut-off of the assay will be replaced by half the value of the cut-off.

95% CIs for GMCs will be based on a back-transformation of student-t CIs for the mean log-transformed anti-HBs antibody concentrations.

4.3.1.2. Main Outputs

The following will be tabulated for each group, and at each timepoint that blood samples are collected for the humoral immune response (anti-HBs antibodies):

- Anti-HBs antibody concentrations (at Day 1, 31, 61 and 361):
  - Total N, GMCs and their 95% CI will be tabulated by study group, as well as summary statistics (median, minimum and maximum)
  - GMCs and related CIs by study group will be represented graphically over time. In addition, GMCs over time combining all groups in a single plot will also be produced.
  - Antibody concentrations will be displayed using reverse cumulative curves for each protocol defined timepoint.
- Seroconversion and seroprotection rates (at Day 31, 61 and 361):
  - Percentage of participants above seroconversion and seroprotection threshold will be tabulated with exact 95% CI for each group at each protocol-specified timepoint.

Note: values in graphs will be displayed on the $\log_{10}$ scale.

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4.5. Safety Analyses

The main safety analyses are described in Section 4.2

4.5.1. Additional Safety Assessments

4.5.1.1. Vital signs

Vital signs (systolic/diastolic blood pressure, and heart rate) and pre-vaccination temperature reported before each study intervention administration (Visit 1 [Day 1], Visit 4 [Day 31] and Visit 8 [Day 161]) will be summarized by group using descriptive statistics.

4.5.1.2. COVID-19 Assessment

The following analyses will be conducted on COVID-19 data:

- Number and incidence of COVID-19 reported as unsolicited AE and SAE with be tabulated by group with exact 95% CI and categorized by MedDRA SOC and PT.
- Severity and outcome of COVID-19 AEs will be tabulated (number and percentage) by group.
- Summary of study completion with reasons for withdrawal related to COVID-19 will be tabulated by group.

4.6. Other Analyses

Not applicable.

4.7. Analyses

The list and sequence of analyses is described in Section 1.2.2

4.8. Changes to Protocol- Defined Analyses

Not applicable.

5. SAMPLE SIZE DETERMINATION

A minimum of 23 (and maximum of 30) participants per group will be enrolled in this study. The target sample size has been reduced from 30 to 23 participants per group for reasons of feasibility, as discussed in Section 4.2.1 of the study protocol. CCI [redacted].

The sample size is not based on formal hypothesis testing and is typical for this kind of study where a treatment is tested for the first-time in human. CCI [redacted]

The primary objectives of the study are to assess the reactogenicity and safety of each vaccine dose throughout the study. CCI [redacted].

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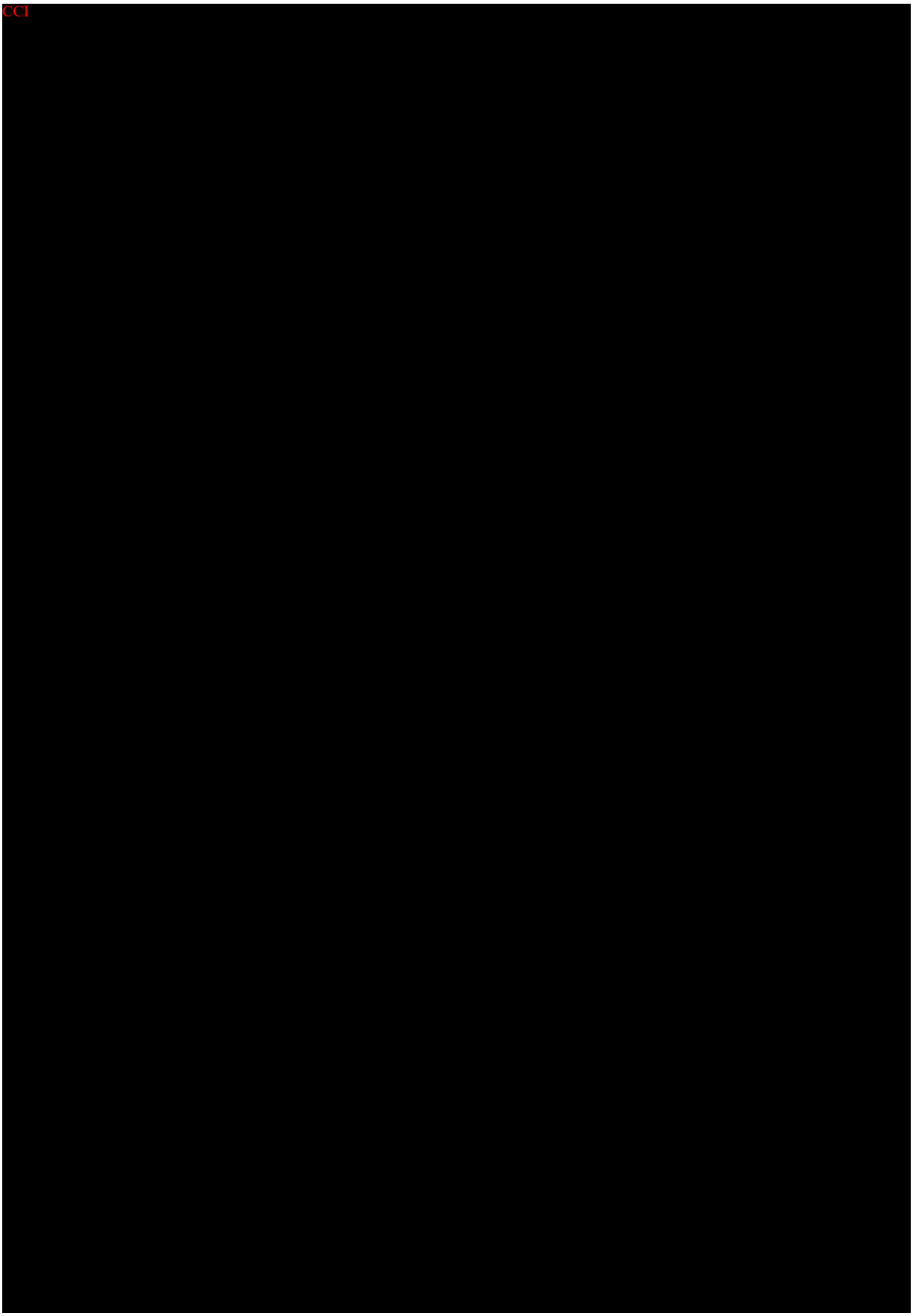

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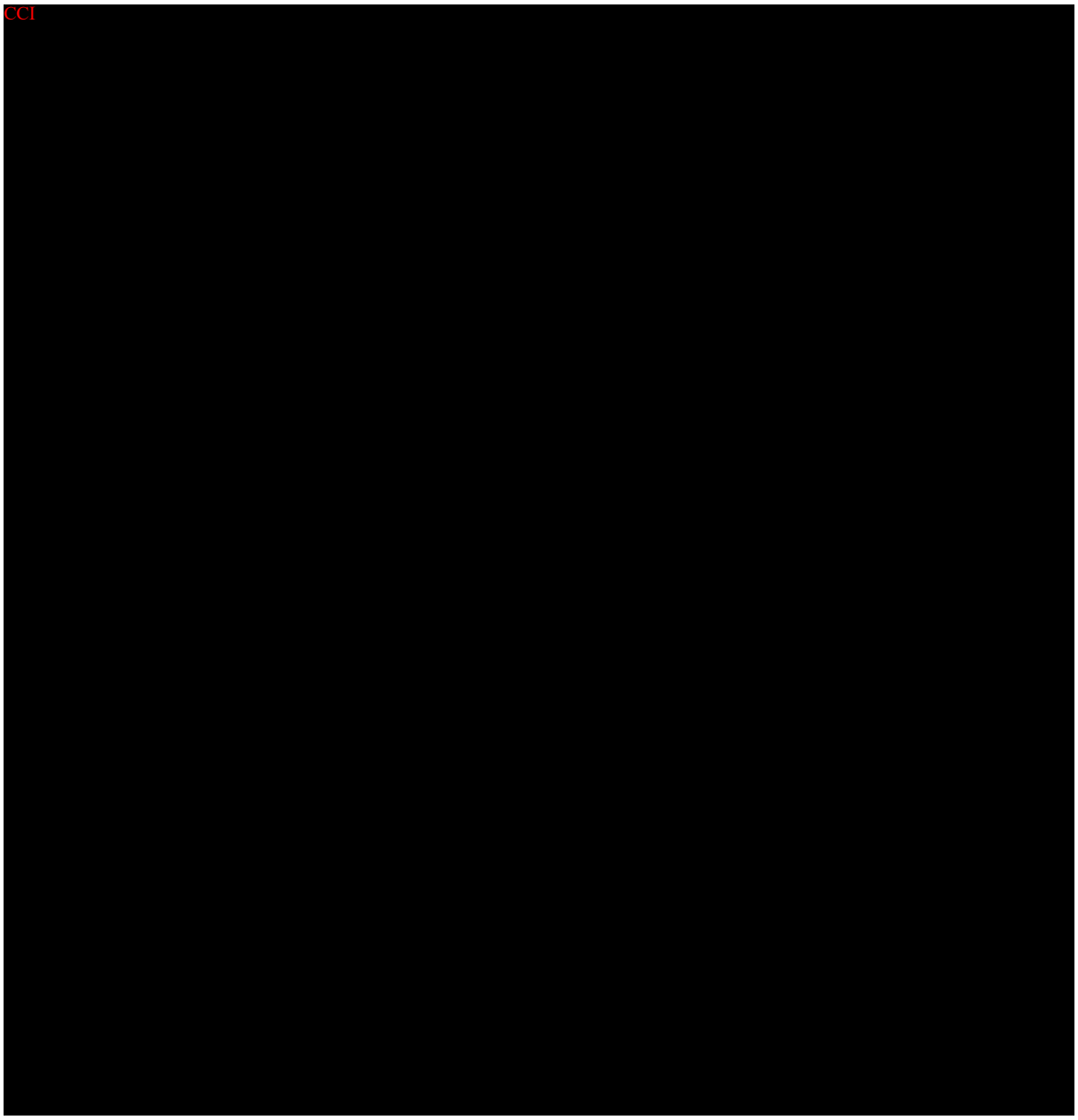

No dropout is assumed for the post-dose 1 evaluation.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

6.1.1. Participant Disposition

The number of participants who are enrolled, vaccinated and in each study group will be tabulated.

In addition, the number of participants in each analysis set will be described, e.g., including the number of participants excluded from the PPS and the number who withdraw and reason for dropout.

6.1.2. Demographic and Baseline Characteristics

N, mean, SD, median, minimum and maximum of age (in years) at first vaccination, height (cm), weight (kg), and body mass index (BMI) (kg/m²) will be computed by group. Participants composition by country, center, sex, race, ethnicity category will be summarized overall and by study group. This analysis will be based on the ES and the PPS.

6.1.3. Participant exposure

The number and percentage of participants who received each study intervention will be tabulated by group and by dose for the ES.

6.1.4. Concomitant Medications

Medications will be coded using the GSKDRUG dictionaries.

The number and percentage of participants using concomitant medication (any medication and any antipyretic) during the 14-day and the 31-day follow-up period after each dose and overall will be tabulated with exact 95% CI.

6.2. Appendix 2 Electronic Clinical Outcome Assessment (eCOA) Compliance

6.2.1. eDiary data

- In this study, solicited events are collected via electronic diary (eDiary).
- When the participant fills in the eDiary, on each day he/she has to fill in all the solicited symptoms in order to be able to submit the daily eDiary entry, during the 14-day follow-up period after each dose.
- Along with the presence of solicited events, participants are required to report symptom intensity. For erythema and swelling, the maximum diameter has to be reported. For temperature, participant has the option of providing the value of temperature in degrees or report it as « Unable to measure ». In both cases, the reporting of temperature will be considered as completed.
- In case of missed entries by the participant, the investigator should contact the participant and report missing data into eCRF. In case of erroneous participant entries, these are corrected via Data Correction Form (DCF) in the e-Diary platform. There was no time constraint for missing data entry or correction foreseen at study start and there is no available information of actual date and time of missing/corrected data collection by the investigator in the database.

- The completion level is defined:
  - Based on eDiary data filled in by the participant (including possible corrections via DCF).
  - Based on data including also information entered by the investigator via missed solicited events page in eCRF.
- For the completion level **within 14-day follow-up period after each dose:**
  - For daily **completion level** calculation:
    - Denominator will be the total number of **eDiaries expected** to be completed by all dosed participants for the given observation day.
    - Numerator will be the number of **eDiaries effectively completed** by the dosed participants for the given observation day.
  - **Overall completion** level for the 14-day follow-up period after each dose:
    - Denominator* will be the total number of eDiaries expected to be completed by dosed participants for the entire 14-day follow-up period after each dose.
    - Numerator will be the number of eDiaries effectively completed by dosed participants during the entire 14-day follow-up period after each dose.

*For the denominator, if the monitoring is performed with data up to 22 May 2024 for example, a participant who received Dose 1 on 20 May is expected to have an eDiary completed for Day 1 and Day 2, but not yet for Day 3 up to Day 14.

- For solicited events **ongoing after the 14-day follow-up period after each dose**, eDiary will continue to be filled for each individual ongoing symptom for each participant. As soon as the intensity of an ongoing symptom is reported as 'None', the symptom is considered as ended.
  - Completion level calculation beyond the solicited follow-up period (15-31 days post each dose):
    - Denominator will be the total number of expected **eDiaries** (i.e., the total number of individual eDiaries for all ongoing symptoms for all participants for symptoms reported on the previous day, Day 14 or beyond).
    - Numerator will be the total number of **eDiaries** that have been completed.

6.2.2. Endpoint Level Completion analysis

The completion level will be measured, daily and overall:

- Within 14-day follow-up period after each doseafter each dose.
- Beyond the solicited follow-up period (i.e., 15-31 days post each dose)

and based on

- eDiary data filled in by the participant (including possible corrections via DCF).
- Data including also information entered by investigator via missed solicited events page in eCRF.

Because on each day participants have to fill in all the solicited symptoms in order to be able to submit the daily eDiary entry, completion level will be identical for all symptoms. The completion level will therefore be summarized once for symptoms, by study group and dose, as well as overall (across the 2 doses), using frequency tables.

The number of participants who have 0 to <50% overall completion level, 50 to <80% overall completion level, and ≥80% overall completion level of eDiary assessments will be summarized too, by study group and dose, as well as overall.

6.2.3. Study level compliance

No study level eCOA compliance will be calculated because the only eCOA foreseen to be followed up in the statistical outputs is the eDiary.

Quality Tolerance Limit on eDiary completion are defined separately from this SAP and are not in scope of this evaluation.

6.3. Appendix 3 Data Derivations Rule

This section contains standard rules for data display and derivation for clinical and epidemiological studies.

6.3.1. Attributing events to vaccine doses

The dose relative to an event is the most recent study dose given to a participant prior to the start of a given event. For example, if the start date of an AE is between Dose 1 and Dose 2, the relative dose will be Dose 1.

If an event starts on the same day as a study dose, the relative dose will be derived from the additional information provided in the eCRF using the contents of the flag indicating if the event occurred before or after study dose. If ‘after study dose’ is selected, the relative dose for the event will be the one administered on the start day of the event. If ‘before study dose’ is selected, the relative dose for the event will be the dose prior to this one.

6.3.2. Handling of missing data

6.3.2.1. Missing data related to immunological outputs (e.g., if a participant misses a visit),

- If there is a small proportion of missing data(<10%), missing or non-evaluable measurements (e.g., for a given participant at a given timepoint) will not be replaced. Therefore, analyses will exclude these participants.

- If there is a large proportion of missing data (≥10%), there may be an assessment to determine if the data are missing due to systematic issues or not (e.g., if not, missing completely at random). The result of this assessment will determine the mitigation strategy (e.g., we may impute data if deemed necessary).

6.3.2.2. Missing covariate data (e.g., missing gender)

- If there is a large proportion of missing data, we will assess if the data are missing due to systemic issues and depending on the results, we may impute data.
- We may also run the models with only individuals that have the full set of data and separately, among all individuals.

6.3.2.3. Dates

When partially completed dates (i.e., dates missing a day and/or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15.
- A missing day and month will be replaced by June 30th.

The following exceptions apply:

When partially completed dates (i.e., with missing day or month) are used in calculations, the following rules will be applied:

- A start date with missing day:
  - If the month is not the same as the vaccine dose, then the imputed start date will be the 1st of the month
  - If the event starts in the same month as the vaccine dose, the flag indicating if the event occurred before or after vaccination (AE.AESTRTPT) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the vaccine dose given during that month. If 'before vaccination' is selected, the imputed date will be one day before the vaccine dose given during that month.
- A start date with missing day and month:
  - If the year is not the same as the vaccine dose, then the imputed start date will be the 1st of January.
  - If the event starts in the same year as the vaccine dose, the flag indicating if the event occurred before or after vaccination (AE.AESTRTPT) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the first vaccine dose given during that year. If 'before vaccination' is selected, the imputed date will be one day before the first vaccine dose given during that year.

- An end date with missing day: the imputed end date will be the last day of the month (30 or 31) or the study conclusion date, whichever comes first.
- An end date with missing day and month: the imputed end date will be the last day of the year (31st of December) or the study conclusion date whichever comes first.

All incomplete concomitant medication/vaccination start/end date will follow the rules above.

6.3.2.4. Laboratory CCI [redacted]

Missing laboratory results (including immunological data) and results from CCI [redacted] will not be replaced.

Unless otherwise specified, results for safety laboratory parameters (excluding immunological data) below the cut-off of the assay will be replaced by the value of the cut-off.

6.3.2.5. Daily recording of solicited events

6.3.2.5.1. Studies with electronic diaries

As this study uses electronic diaries for the collection of solicited AEs, a solicited AE will be considered present only when a daily recording of grade 1 or more is present. Missing or non-evaluable measurements will not be replaced.

6.3.2.6. Unsolicited adverse events

Unsolicited AE summaries are including SAEs unless specified otherwise.

Missing severity, relationship with study vaccine, and outcome of unsolicited AEs will not be replaced and will appear as 'UNKNOWN' in all statistical output.

6.3.3. Data derivation

6.3.3.1. Age at first dose in years

Age will be calculated as the number of years between the date of birth and the date of first vaccination.

In case of partial dates, the following 2 dates will be used as replacement dates:

- 15th of month, if the day is missing.
- 30th of June, if day and month are missing.

6.3.3.2. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

6.3.3.3. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
"NEG", "-", or "(-)"	cut-off/2
"POS", "+", or "(+)"	cut-off
"< value" and value is <= assay cut-off	cut-off/2
"< value" and value is > assay cut-off	value
"> value" and value is < assay cut-off	cut-off/2
"> value" and value is >= assay cut-off	value
"value" and value is < cut-off	cut-off/2
"value" and value is >= cut-off	Value
"value" and value is > ULOQ	ULOQ
All other cases	missing

6.3.3.4. Geometric mean titers (GMTs) and concentrations (GMCs)

Geometric Mean Titer (GMT) or GMC calculations are performed by taking the inverse logarithm of the mean of the log10-titer or concentration transformations. Non quantifiable antibody titers or concentrations will be converted as described in Section 6.3.3.3 for the purpose of GMT/GMC calculation. Cut-off values are defined by the laboratory before the analysis.

6.3.3.5. Onset day

The onset day for an event (e.g. AE, concomitant medication/vaccination) is the number of days between the last (i.e., most recently received) study dose (i.e., it may be dose 1 or dose 2 of the vaccine) and the start date of the event plus one day. This is 1 for an event occurring on the same day as a study dose (and reported as starting after study dose) and e.g., 2 for an event occurring on the day after the study dose was given.

6.3.3.6. Duration of events

The duration of an event with a start and end date will be the difference between the start and end date plus one day, i.e. an event that starts on 3 March 2018 and ends on 12 March 2018 has a duration of 10 days.

The duration of solicited events will be calculated as the sum of the individual days with the AE reported at grade 1 or higher during the solicited event period as well as time between the first and the last day of symptoms.

6.3.3.6.1. Counting rules for combining solicited and unsolicited adverse events

For output combining solicited and unsolicited AEs, all SAEs will be considered systemic events since the administration site flag is not included in the expedited AE eCRF pages. Unsolicited AEs with missing administration site flag will also be considered systemic.

Multiple events with the same PT which start on the same day are counted as only one occurrence.

6.3.3.7. Counting rules for occurrences of solicited events

When the occurrences of solicited events are summarized, each event recorded as having occurred during a specific period will be counted as only one occurrence regardless of the number of days on which it occurs.

Table 11 Intensity grading scale for solicited events

Event	Intensity grade	Parameter
Pain at administration site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal everyday activities.
	2	Moderate: Painful when limb is moved and interferes with everyday activities.
	3	Severe: Significant pain at rest. Prevents normal everyday activities.
Redness at administration site	0	< 25 mm
	1	25 - 50 mm
	2	51 - 100 mm
	3	> 100 mm
Swelling at administration site	0	< 25 mm
	1	25 - 50 mm
	2	51 - 100 mm
	3	> 100 mm
Temperature*	0	< 38.0°C (< 100.4°F)
	1	38.0°C (100.4°F) - 38.4°C (101.1°F)
	2	38.5°C (101.2°F) - 38.9°C (102.0°F)
	3	> 38.9°C (> 102.0°F)
Headache Fatigue Myalgia Arthralgia Chills Malaise	0	None
	1	Mild: Symptom that is easily tolerated
	2	Moderate: Symptom that interferes with normal activity
	3	Severe: Symptom that prevents normal activity
Loss of appetite	0	None

Event	Intensity grade	Parameter
	1	Mild: Loss of appetite without decreased oral intake
	2	Moderate: Loss of appetite with decreased oral intake but without weight loss
	3	Severe: Loss of appetite with decreased oral intake and weight loss
Nausea	0	None
	1	Mild: Nausea present but not interfering with oral intake
	2	Moderate: Nausea leading to decreased oral intake
	3	Severe: Nausea leading to minimal to no oral intake
Vomiting	0	None
	1	Mild: 1 - 2 episodes/24 hours
	2	Moderate: 2 episodes/24 hours
	3	Severe: Requires outpatient IV hydration
Diarrhea	0	None
	1	2 - 3 loose stools/24 hours
	2	4 - 5 loose stools/24 hours
	3	6 or more watery stools/24 hours or requires outpatient IV hydration

IV = intra venous

*The route for measuring temperature can be oral, axillary or tympanic. Fever is defined as temperature ≥38.0°C regardless of the location of measurement.

6.3.3.8. Counting rules for occurrence of unsolicited adverse events

Unsolicited AE summaries are including SAEs unless specified otherwise.

As per CDISC Vaccines Therapeutic Area guide, the solicited events which continue beyond the observation period are stored in the AEs domain but they do not contribute to the summaries of unsolicited AEs.

Missing severity, relationship with study vaccine, and outcome of unsolicited AEs will not be replaced and will appear as 'UNKNOWN' when displayed in a statistical output.

6.3.4. Display of decimals

6.3.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with one decimal except for 100% in which case no decimal will be displayed.

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6.3.4.3. Demographic/baseline characteristics statistics

The mean, median, and SD for continuous baseline characteristics (height, weight, BMI, pre-dose body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maximum and minimum of transformed body temperatures will be displayed with one decimal.

6.3.4.4. Serological summary statistics

For each assay, GMTs or GMCs and their confidence limits will be presented with one decimal, as well as GMT/GMC fold increase from pre-dose.

GMT or GMC group ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

7. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

CCI [redacted]

Fisher, RA, *Statistical methods for research workers, 5th edn*. Oliver & Boyd, Edinburgh. 1934

CCI [redacted]

CCI [redacted]

CCI [redacted]