NCT05513053

SAP Core Body

Title: Immunogenicity and Safety of Quadrivalent Recombinant Influenza Vaccine (RIV4) in Children and Adolescents Aged 9 to 17 Years and Adults Aged 18 to 49 Years.

Study Code: VAP00027

Study Phase: Phase III

SAP Core Body Version: 2.0

SAP Core Body Date: 6 December-2023

Protocol Version Number: 2.0

Version History

Previous Version(s)	Date	Comments
1.0	21 April 2022	
2.0	6 December 2023	Addition of a complementary analysis to assess the difference in enrolment period between age groups. Clarification of statistical blood visit window. Clarification on conducting the supportive statistical analyses on the Full Analysis Set (FAS) population, if the attrition rate from FAS to the Per Protocol Analysis Set (PPAS) is greater than 10%.

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1 Overall Design

The design of the study is summarized in Table 1.1.

Table 1.1: Overall design

Type of design	Parallel, multi-center
Phase	III
Control method	None
Study population	Healthy participants aged 9 to 49 years
Level and method of blinding	Open-label
Study intervention assignment method	Maximization of planned stratification of each age subgroup
Number of participants	Approximately 1334 participants (667 children and adolescents 9 to 17 years of age, and 667 adults18 to 49 years of age)
Intervention groups	Eligible participants will receive a single injection of RIV4 at D01
Total duration of study participation	Approximately 6 months
Countries	US and Europe
Use of an Independent Data Monitoring Committee, Dose Escalation Committee, or similar review group	Safety Management Team (SMT)

Table 1.2: Schedule of activities (modified compared to protocol)

Phase III Study, 2 Visits, 2 Phone calls, 1 Vaccination, 2 Blood Samples, 6 Months' Duration Per Participant

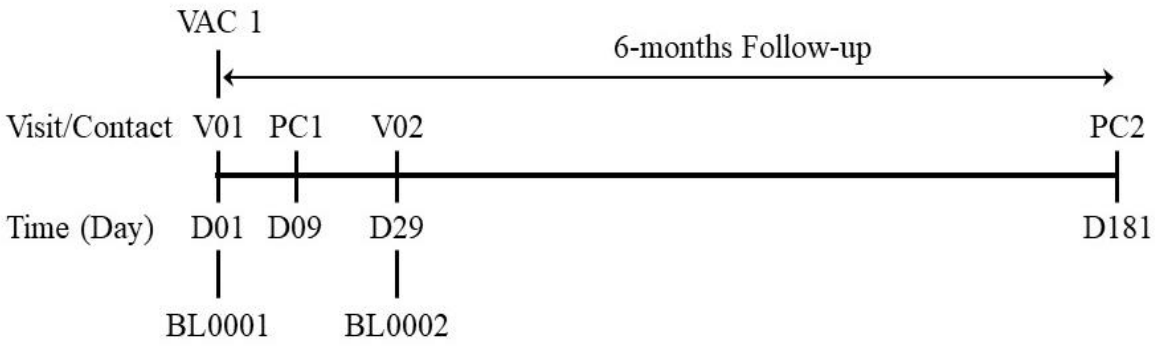
Visit/Contact	***Collection of information in the case report form (CRF)***	**Visit 1**	**Phone Call (PC) 1***	**Visit 2**	**PC2 6-month safety follow-up†**
Study timelines (Days)		D01	D09	D29	D181
Time interval (Days)			V01 + 8D	V01 + 28D	V01 + 180D
Time windows (Days)		NA	[+2 D]	[-2, +7 D]	[+14 D]
Visit procedures:					
Informed consent	X	X			
Inclusion/exclusion criteria	X	X			

Visit/Contact	***Collection of information in the case report form (CRF)***	**Visit 1**	**Phone Call (PC) 1***	**Visit 2**	**PC2 6-month safety follow-up†**
Study timelines (Days)		D01	D09	D29	D181
Time interval (Days)			V01 + 8D	V01 + 28D	V01 + 180D
Time windows (Days)		NA	[+2 D]	[-2, +7 D]	[+14 D]
Visit procedures:					
Collection of demographic data§§	X	X			
Urine pregnancy test (if applicable) ‡		X			
Collection of Medical history (Significant medical history)***	X	X			
Collection of concomitant medications	X Reportable concomitant medication	28 days after vaccination			
History of seasonal influenza vaccination	X	X			
Contact Interactive Response Technology (IRT) system for participant number, dose number and randomization to the SN subset	X	X			
Blood sampling (BL) (5 mL)	X	BL0001**		BL0002	
Vaccination (VAC)	X	X			
Immediate surveillance (30 min)	X	X			
Collection of solicited injection site and systemic reactions	X	7 days after vaccination			
Collection of unsolicited adverse events (AEs)	X	28 days after vaccination			
Collection of medically attended AEs (MAAEs)	X	28 days after vaccination			
Collection of information on serious adverse events (SAEs), including adverse events of special interest (AESIs)	X	To be reported at any time during the study			

Visit/Contact	***Collection of information in the case report form (CRF)***	**Visit 1**	**Phone Call (PC) 1***	**Visit 2**	**PC2 6-month safety follow-up†**
Study timelines (Days)		D01	D09	D29	D181
Time interval (Days)			V01 + 8D	V01 + 28D	V01 + 180D
Time windows (Days)		NA	[+2 D]	[-2, +7 D]	[+14 D]
Visit procedures:					
Collection of pregnancy	X	To be reported at any time during the study			
End of Active Phase participation record	X			X	
Six months follow-up participant record	X				X

* The investigator or an authorized designee will remind the participant or participant's parent(s)/legally acceptable representative(s) to bring back the DC/eDC at the next visit and will answer any questions.
† The investigator or an authorized designee will interview the participant or participant's parent(s)/legally acceptable representative(s) to collect the information recorded in the MA, and will attempt to clarify anything that is unclear
‡To be performed in menarche participants
** Blood sample to be drawn before vaccination
§§ To comply with US Food and Drug Administration (FDA) expectations, Sponsors are to enroll participants who reflect the demographic for clinically relevant populations with regards to age, gender, race, and ethnicity (FDA. Collection of race and ethnicity data in clinical trials: Guidance for industry and Food and Drug Administration staff [Internet]. 2016. Available from: https://www.fda.gov/media/75453/download)
***Including history of laboratory-confirmed influenza illness over the previous 3 influenza seasons.

Figure 1.1: Graphical study design



BL: Blood sample
VAC: vaccination
PC: Phone Call

Detailed design is provided in Sections 4.1 and 1.1 of the protocol.

2 Objectives and Endpoints

Table 2.1: Objectives and endpoints

Objectives	Endpoints
Primary	
• To demonstrate the non-inferior hemagglutination inhibition (HAI) immune response of quadrivalent recombinant influenza vaccine (RIV4) for the 4 strains in participants aged 9 to 17 years vs participants aged 18 to 49 years	• Individual HAI titer 28 days after vaccination (D29) • Seroconversion (SC) (titer < 10 [1/dil] at D01 and post-injection titer ≥ 40 [1/dil] at D29, or titer ≥ 10 [1/dil] at D01 and a ≥ 4-fold rise in titer [1/dil] at D29)
Key Secondary	
Immunogenicity • To summarize the HAI immune response induced by RIV4 in all participants.	• Individual-HAI titer on D01 and 28 days after vaccination (D29) • Detectable HAI titer, ie, with a titer ≥ 10 (1/dil) at D01 and 28 days after vaccination (D29) • Individual titer ratio: 28 days after vaccination (D29) /D01 • Participants with titer ≥ 40 (1/dil) on D01 and 28 days after vaccination (D29) • SC (titer < 10 [1/dil] at D01 and post-injection titer ≥ 40 (1/dil) at D29 or titer ≥ 10 (1/dil) at D01 and a ≥ 4-fold rise in titer (1/dil) at D29)
Safety • To describe the safety profile of RIV4 vaccine in all participants and by age group	• Occurrence of any unsolicited systemic adverse events (AEs) reported in the 30 minutes after vaccination • Occurrence of solicited (pre-listed in the participant's diary card [DC]/electronic diary card [eDC] and case report book [CRB]) injection site reactions and systemic reactions occurring up to 8 days after vaccination • Occurrence of unsolicited AEs up to 28 days after vaccination

	• Occurrence of medically attended adverse events (MAAEs) up to 28 days after vaccination • Occurrence of serious adverse events (SAEs) (including adverse events of special interest [AESIs]) throughout the study • Occurrence of AESIs throughout the study
Exploratory	
• To describe the neutralizing antibody immune response in a subset of participants1.	• Individual seroneutralization (SN) antibody (Ab) titer on D01 and 28 days after vaccination (D29) • Individual SN Ab titer ratio (fold increase in post-vaccination titer relative to D01) at 28 days after vaccination (D29) • Participants with SN Ab titers ≥ 20 (1/dil), ≥ 40 (1/dil), ≥ 80 (1/dil) at 28 days after vaccination (D29) • Fold-increase in SN Ab titer (post/pre) ≥ 2 and ≥ 4 at 28 days after vaccination (D29) • Detectable SN Ab titer (SN Ab titer ≥ 10 [1/dil]) at D01 and 28 days after vaccination (D29)

[1] At least 400 participants will be randomized to be included in the subset. The randomization of participants in the subset will be stratified to include at least 30% of children aged 9 to 11 years among children and adolescents 9 to 17 years old; and the proportion of adults aged above 35 years will be limited to 50% in the study group 18 to 49 years.

3 Statistical Considerations

3.1 Statistical Hypotheses

Primary Objective:

Statistical methodology for analyzing the 8 primary endpoints (geometric mean titers [GMTs] and SC rates).

Non-inferiority (NI) of the age group 9-17 years as compared to 18-49 years after vaccination of both age groups with RIV4, will be conducted for GMTs and SC rates.

Since all 8 hypotheses have to be rejected at 0.025 significance level, no formal adjustment for multiplicity is necessary.

For each strain, the NI methodology will be applied to compare the post-vaccination GMTs and the SC rates between the study groups using a 1-sided Type I error rate of 0.025 with the given individual hypothesis.

The primary analysis will be conducted in 2 steps starting with testing for NI of GMTs between the age group 9-17 years and the age group 18-49 years. If NI of GMTs based on the 4 strains is demonstrated, then NI of the SC rates will be also tested.

Step1: Geometric Mean Titers

For each of the 4 strains, the null hypothesis and the alternative hypothesis are:

H_0: $GMT_{RIV4(9\text{-}17y)} / GMT_{RIV4(18\text{-}49y)} \leq 0.667$

H_A: $GMT_{RIV4(9\text{-}17y)} / GMT_{RIV4(18\text{-}49y)} > 0.667$

or equivalently,

$$H_0: \log_{10}(GMT_{RIV4(9\text{-}17y)}) - \log_{10}(GMT_{RIV4(18\text{-}49y)}) \leq \log_{10}(0.667)$$

$$H_A: \log_{10}(GMT_{RIV4(9\text{-}17y)}) - \log_{10}(GMT_{RIV4(18\text{-}49y)}) > \log_{10}(0.667)$$

For the separately considered GMT hypotheses, if the null hypothesis is rejected, then the alternative hypothesis of NI is supported.

All 4 strains must show NI of GMTs to consider that GMTs have demonstrated NI.

Step 2: Seroconversion Rates

For each of the 4 strains, the null hypothesis and the alternative hypothesis are:

H_0: $P_{RIV4(9\text{-}17y)} - P_{RIV4(18\text{-}49y)} \leq -10\%$

H_A: $P_{RIV4(9\text{-}17y)} - P_{RIV4(18\text{-}49y)} > -10\%$

For the separately considered seroconversion hypotheses, if the null hypothesis is rejected, then the alternative hypothesis of NI is supported.

All 4 strains must demonstrate NI of the SC rates to consider that SC rates have demonstrated NI.

Secondary Objectives:

There are no statistical hypotheses to be tested in the secondary objectives.

3.2 Sample Size Determination

A total of approximately 1200 evaluable participants 9 to 49 years of age (600 children and adolescents 9 to 17 years of age [approximately 30% children 9 to 11 years of age] and 600 adults 18 to 49 years of age) will be assessed.

Assuming the same GMT for each strain in the age groups (9 to 17 years vs. 18 to 49 years) compared, and a standard deviation of $\log_{10}$ titers of 0.6 with a NI margin of 1.5; NI for GMTs would be demonstrated with a power of at least 99.6%.

Assuming in each vaccine group the same expected SC rates (0.7, 0.5, 0.6, 0.5) for each of the 4 strains (A/H1N1, A/H3N2, B/Yamagata and B/Victoria), based on conservative estimates from historical data, and a NI margin of 10%, the NI for SC rate can be demonstrated with a study power of approximately 80.10% (96.66%, 93.70%, 94.38% and 93.70% for each strain, respectively).

Hence, the overall study power is estimated to be 80.0% (80.0% = 99.6%[GMTs] x 80.10%[SC rate]).

As shown above, to keep the overall study power of 80%, the sample size was increased accordingly, to have an overall type II error <20% for the 8 NI tests.

Assuming an attrition rate of approximately 10% in this age group, a total of approximately 1334 participants 9 to 49 years of age will be enrolled.

3.3 Populations for Analyses

For the purposes of analysis, the following analysis sets will be defined:

Participant Analysis Set	Description
Enrolled	All participants with data in the CRF. Note: the study groups are not randomized. The study groups are determined based on age. However, a subset of participants from each age group will be randomly selected in order for their blood to be tested using the SN method.
Safety Analysis Set (SafAS)	Participants who have received one dose of the study vaccine. Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).
Full analysis set (FAS)	Subset of participants who received one dose of the study vaccine and had a post-vaccination blood sample. For the assessment of the immune response by SN assay, the analysis will be performed on the participants from FAS who were randomized in the exploratory subset (FAS-SN).

Per-protocol analysis set (PPAS)	Subset of the FAS. Participants presenting with at least one of the following criteria will be excluded from the PPAS: · Participant did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria · Participant did not receive vaccine in the proper time window · Preparation and / or administration of vaccine was not done as per-protocol · Participant did not provide the post-dose serology sample at V02 in the proper time window ([D26, D39]) or a post-dose serology sample was not drawn · Participant received a protocol-prohibited medications impacting or that may have an impact on the immune response · Any other deviation identified during the study conduct and identified as relevant by the clinical team during data review, ie, indicated as excluding subjects from this analysis set in the manual deviations dataset. This will be limited to exceptional circumstances given the open-label nature of the trial. For the assessment of the immune response by SN assay, the analysis will be performed on the participants from PPAS who were randomized in the exploratory subset (PPAS-SN).

3.4 Statistical Analyses

3.4.1 General Considerations

All statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics Platform using the SAS® software, Version 9.4 or above (SAS Institute, Cary, North Carolina, USA).

For descriptive purposes, the following statistics will be presented:

Table 3.1: Descriptive statistics produced

Disposition and follow-up description	**Categorical data**	At least number of participants (Percentage of participants are also possible).
	Continuous data	Mean, standard deviation, minimum and maximum.
Baseline characteristics	**Categorical data**	Number of participants. Percentage of participants.
	Continuous data	Mean, standard deviation, quartiles, minimum and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% confidence intervals [CIs] for main endpoints) of participants.

		Unsolicited: Number and percentage (95% CIs for main endpoints) of participants and number of events.
Immunogenicity results	**Categorical data (seroconversion, cutoff)**	Number and percentage (95% CIs for main endpoints) of participants.
	Continuous data (titer / concentration)	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1), ie, using the inverse of the beta integral with SAS®.

For immunogenicity results, assuming that $\log_{10}$ transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on $\log_{10}$ (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.

3.4.2 Primary Endpoints

The immunogenicity parameters will be calculated in each study group with their 95% CIs using the exact binomial distribution (Clopper-Pearson method) for proportions and using normal approximation of log-transformed for GMTs and GMTs ratio.

Statistical methodology for analyzing the 8 primary endpoints (GMTs and SC rates).

NI of RIV4 in participants aged 9 to 17 years vs participants aged 18 to 49 years will be conducted for GMTs and SC rates.

For each strain, the NI methodology will be applied to compare the post-vaccination GMTs and the SC rates between the groups using a 1-sided Type I error rate of 0.025 with the given individual hypothesis.

The primary analysis will be conducted in 2 steps starting with testing for NI of GMTs between the age group 9-17 years and the age group 18-49 years. If NI of GMTs based on the 4 strains is demonstrated, then NI of the SC rates will be also tested.

Step 1: Geometric Mean Titers

Assuming that $\log_{10}$ transformation of the data follows a normal distribution, the $\log_{10}$ (data) will be used for the statistical analysis, then antilog transformations will be applied to the results of calculations, in order to provide the results in terms of geometric means (GMs).

The statistical methodology is based on a 2-sided 95% CI of the ratio of the GMTs (RIV4[9-17y] divided by RIV4[18-49y]) at 28 days after vaccination. NI for GMTs is demonstrated if the lower

limit of the 2-sided 95% CI of the GMT ratio is > 0.667 for each of the 4 virus strains. The 95% CI will be calculated using normal approximation of log-transformed titers.

Step 2: Seroconversion Rates

The statistical methodology is based on a 2-sided 95% CI of the difference in SC rates (RIV4 [9-17years] minus RIV4 [18-49years]) at 28 days after vaccination. NI for SC rates is demonstrated if the lower limit of the 2-sided 95% CI is >-10% for the 4 strains. The 95% CI of the rate difference is computed using the Wilson Score method without continuity correction.

All 4 strains must demonstrate NI of the SC rate in order for study SC rates to demonstrate NI success.

The PPAS will be used as the primary analysis set for this objective (GMTs and SC rates).

The primary objective is successful if NI for GMTs and NI for SC rates are successful.

3.4.3 Secondary Endpoints

Immunogenicity

The immunogenicity parameters will be calculated with their 95% CIs using the exact binomial distribution (Clopper-Pearson method) for proportions and using normal approximation of log-transformed for GMTs and GMTs ratio. The 95% CI of proportions difference (ie, difference between vaccine groups in SC) will be calculated using Wilson Score method without continuity correction. All analyses will be conducted by study group.

The following descriptive statistics will be displayed:

- GMTs of individual-HAI titer on D01 and 28 days after vaccination (D29)
- Percentage of participants with detectable HAI titer, ie, with a titer ≥ 10 (1/dil) at D01 and 28 days after vaccination (D29)
- GMs of individual titer ratio: 28 days after vaccination (D29) /D01
- Percentage of participants with titer ≥ 40 (1/dil) on D01 and 28 days after vaccination (D29)
- SC rates (titer < 10 [1/dil] at D01 and post-injection titer ≥ 40 (1/dil) at D29 or titer ≥ 10 (1/dil) at D01 and a ≥ 4-fold rise in titer (1/dil) at D29)
- The RCDCs of pre-vaccination titer (D01), and post-vaccination titer (D29) will be generated for each study group. The RCDCs will include the plots of the 2 study groups the same figure.

The analysis will be conducted for each immunogenicity variable on the PPAS, and on FAS if the attrition rate from FAS to PPAS is greater than 10%.

In addition, subgroup analyses will be performed; in particular, immunogenicity will be described according to age subgroups (9-11y, 12-17y, 18-34y and 35-49y), sex, race, previous influenza vaccination status (received a seasonal influenza vaccine in the last past influenza season or not) and baseline seropositivity status (seropositive and seronegative are defined as baseline antibody titer ≥ 1:10 or < 1:10)., as appropriate according to number of participants in the respective subgroups.

Safety

For the main safety parameters, 95% CIs of point estimates will be calculated using the exact binomial method (Clopper-Pearson method) for single proportions and using the normal approximation for quantitative data.

All analyses will be descriptive; no hypotheses will be tested.

The number of participants with documented safety will be used as denominator of the frequencies.

For solicited reactions, the denominator will be the total number of participants who have non-missing data for the endpoint considered

For unsolicited AEs, the denominator will be the total number of participants who were vaccinated.

SafAS will be the analysis population of safety data.

In terms of contents, solicited reactions will be presented by time to onset, maximum severity, number of days of occurrence and action taken; unsolicited AEs will be presented by causal relationship, time of onset, maximum severity and duration; SAEs will be presented by causal relationship, seriousness and outcome.

Subgroup analyses will also be performed; in particular, the main safety endpoints will be described according to age subgroups (9-11y, 12-17y, 18-34y and 35-49y), previous vaccination status, sex and race, as appropriate according to number of participants in the respective subgroups.

3.4.4 Exploratory Endpoints

The immunogenicity parameters will be calculated with their 95% CIs using the exact binomial distribution (Clopper-Pearson method) for proportions and using normal approximation of log-transformed for GMTs. All analyses will be conducted by study group. The main parameters will also de described by age subgroup. For some parameters (eg. GMTs, 4-fold rise) difference or ratio between groups may be calculated with 95%CI. The 95% CI of ratios of GMTs will be calculated using normal approximation of log-transformed titers and those for proportions difference (ie, 4-fold rise) will be calculated using Wilson Score method without continuity correction.

Neutralizing Ab titers will be measured for each influenza strain with the SN method.

In particular, the following endpoints will be described with 95% CIs:

- GMTs of individual SN antibody (Ab) titer on D01 and 28 days after vaccination (D29)
- GMs of individual SN Ab titer ratio (fold increase in post-vaccination titer relative to D01) at 28 days after vaccination (D29)
- Percentage of participants with SN Ab titers ≥ 20 (1/dil), ≥ 40 (1/dil), ≥ 80 (1/dil) at 28 days after vaccination (D29)

- Percentage of participants with fold-increase in SN Ab titer (post/pre) ≥ 2 and ≥ 4 at 28 days after vaccination (D29)
- Percentage of participants with detectable SN Ab titer (SN Ab titer ≥ 10 [1/dil]) at D01 and 28 days after vaccination (D29)
- The RCDCs of pre-vaccination titer (D01) and post-vaccination titer (D29) will be generated for each study group. The RCDCs will include the plots of the 2 groups on the same figure.

In addition, the descriptive summary of SN immunogenicity results will also be produced by:

- Serological SN status at baseline (< 10 and ≥ 10 1/dil)
- Previous influenza vaccination status
- Age subgroup
- Sex
- Race

The analysis will be conducted for each immunogenicity variable on the PPAS subset (PPAS-SN) and on the FAS subset (FAS-SN) if the attrition rate from FAS-SN to PPAS-SN is greater than 10%.

3.4.5 Handling of Missing Data and Outliers

3.4.5.1 Safety

Generally, no replacement will be done for Safety Missing Data and Outliers.

3.4.5.1.1 Immediate

For unsolicited systemic AEs, a missing response to the “Immediate” field is assumed to have occurred after the 30-minute surveillance period and will not be imputed.

3.4.5.1.2 Causal Relationship

By convention, all events reported at the injection site (either solicited or unsolicited) will be considered as related to the administered product and then referred to as reactions. In a same way, all solicited systemic events pre-listed in the CRF are also considered as related to vaccination and will be considered as reactions.

- For unsolicited systemic AE, missing relationship will be considered as related to study vaccine(s) at the time of analysis.
- The missing relationship to study procedures for SAEs will not be imputed.

3.4.5.1.3 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.2.1.1.1. For unsolicited AEs, missing intensities will remain missing and will not be imputed.

3.4.5.1.4 Start Date and End Date

Missing or partially missing start dates or end dates for unsolicited AEs (including SAEs) will remain missing and not be imputed. If the start date is missing or partially missing, the time of onset will be considered missing. If either the start date or end date is missing or partially missing, the duration will be considered missing.

Missing or partially missing end dates for ongoing solicited AEs will remain missing and not be imputed.

3.4.5.1.5 Action Taken

Missing actions taken will remain missing and not be imputed.

3.4.5.2 Immunogenicity

No imputation of missing values and no search for outliers will be performed. LLOQ and ULOQ management will be performed as described in Section 4.2.3.1.

3.5 Interim Analysis

The statistical analysis will be performed as follows:

- An interim analysis on immunogenicity focused on the HAI data to address the primary objective and safety results obtained on data collected within 28 days after vaccination (from D01 to D29).
- A final analysis will be performed after the SN data are available and the 6-month safety follow-up data have been collected.

No statistical adjustment for the interim analysis is necessary because there are no repeat analyses of the same hypotheses.

3.6 Data Monitoring Committee (DMC)

No independent Data Monitoring Committee (DMC) is planned.

Participant safety data will be continuously monitored by the Sponsor's internal safety management team (SMT), led by the Global Safety Officer, to detect any safety signals during the study period.

In addition, this study will include an early safety data review (ESDR), when at least 10% of subjects have been vaccinated and provided safety data for 7 days after vaccination.

A separate SAP will be dedicated to this analysis.

4 Complementary Information on Assessment Methods

4.1 Complementary Information for Endpoints Assessment Methods

Not applicable.

4.2 Complementary Information on Derived Endpoints: Calculation Methods

4.2.1 Safety

4.2.1.1 Solicited Reactions

4.2.1.1.1 Daily Intensity

All daily records for solicited reactions will be derived into daily intensity according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing (Unknown).

For the derivation of daily intensities, the following sequential steps will be applied:

1) Solicited reactions (except Fever/Pyrexia) with CRF presence recorded as "No" and with all daily records missing (Unknown) then all daily intensities will be derived as None.
2) For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in the protocol; this assumes a reaction that is too large to measure (non-measurable, "NM") is Grade 3. Note: the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults).

Note: The maximum intensity on the ongoing period is derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

4.2.1.1.2 Maximum Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 4.2.1.1.1 and is calculated as the maximum of the daily intensities over the period considered.

4.2.1.1.3 Presence

Presence is derived from the maximum overall intensity over the time period considered:

- None: No presence
- Grade 1, Grade 2, or Grade 3: Presence

- Missing or Unknown: Missing presence

Participants with at least one non-missing presence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing presence will not be included in the analysis of the endpoint.

4.2.1.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.2.1.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., reaction occurs over two separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

Time of onset period is displayed as, D01-D04, D05-D09.

4.2.1.1.5 Number of Days of Occurrence During the Solicited Period

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.2.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of presence on the solicited period with a specified intensity may also be derived.

4.2.1.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of presence is derived from the daily intensities and the end date of the reaction after the end of the solicited period. The overall number of days of presence is:

- (End date - vaccination date) + (number of days of presence within the solicited period) - length of the solicited period + 1

If the end date is missing or incomplete (contains missing data), the overall number of days of presence will be considered as Missing.

4.2.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.2.1.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement will determine the ongoing status of the reaction.

- Ongoing: if the last daily intensity of the solicited period is at least Grade 1 and the maximum intensity on the ongoing period is at least Grade 1
- Not ongoing: if the last daily intensity of the solicited period is None or the maximum intensity on the ongoing period is None.
- Missing: all other conditions (in this case, it is not included in the denominator of the ongoing analysis in the safety tables).

4.2.1.2 Unsolicited AEs

4.2.1.2.1 Presence

An observation will be considered an event if it has at least a verbatim term and is not a Grade 0 intensity event.

Grade 0 events are not included in safety analysis but are included in separate listings.

4.2.1.2.2 Intensity

Intensity will be derived according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing

If the unsolicited AE is measurable and its preferred term is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule of the intensity scales defined in the protocol for that measurable injection site or systemic reaction. Note the intensity could be considered as "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement >0 mm but < 25 mm in adults)

Intensity for the other unsolicited AEs will correspond to the value reported in the CRF.

The maximum intensity corresponds to the highest intensity for a unique term.

4.2.1.2.3 Time of Onset

Time of onset is derived from the start date of the unsolicited AE and the date of vaccination:

Time of Onset = start date of the unsolicited AE - date of vaccination + 1 (if D01 is the first vaccination day).

The time of onset is considered as missing only if one or both dates are missing or partially missing.

The unsolicited AEs will be analyzed "Within 28 days" after vaccination, which corresponds to AEs with a time of onset between D01 and D29 or missing. An AE with missing time of onset will be considered to have occurred just after the vaccination, so will be included in these tables.

Time of onset period is displayed as D01-D04, D05-D08, D09-D15, D16 or later, and Missing.

Note: Unsolicited AE that occurred before vaccination (negative time of onset) or with a time of onset higher than defined above will not be included in analysis but will be listed separately.

4.2.1.2.4 Duration

Duration is derived from the start and end dates of the unsolicited AE:

- Duration = End date of unsolicited AE - start date of unsolicited AE + 1.

The duration is considered as missing only if one or both of the start and end dates of the unsolicited AE is missing or partially missing.

4.2.1.2.5 Medically-Attended Adverse Event

An event will be considered as an MAAE if "Yes" is checked for "Is the event an MAAE?" in the CRF. MAAE will be analyzed within 28 days after vaccination, from D29 to 180 days after vaccination, and within 180 days after vaccination.

4.2.1.2.6 Serious Adverse Events

An event will be considered as a serious event if "Yes" is checked for "Serious" in the CRF.

SAEs will be analyzed within 28 days after vaccination, from D29 to 180 days after vaccination, and within 180 days after vaccination.

4.2.1.2.7 Adverse Events of Special Interest

An event will be considered as an AESI if "Yes' is checked for "Is the event an AESI?" in the CRF.

AESIs will be analyzed within 28 days after vaccination, from D29 to 180 days after vaccination, and within 180 days after vaccination.

4.2.2 Other Safety Endpoints

4.2.2.1 Pregnancy

This information will be summarized as collected. No derivation or imputation will be done.

4.2.2.2 Action Taken

This information will be summarized as collected, including missing observations. No derivation or imputation will be done.

4.2.2.3 Seriousness

This information will be summarized as collected. No derivation or imputation will be done.

4.2.2.4 Outcome

This information will be summarized as collected. No derivation or imputation will be done.

4.2.2.5 Causal Relationship

This information will be summarized as collected in the field "Relationship to study vaccine". Missing causal relationship will be handled as described in Section 3.4.4.1.2. Relationship to study procedure is only presented in the listing.

4.2.2.6 Adverse Events Leading to Study Discontinuation

This information will be summarized as collected. A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation before the end of active phase.

In general, the items that are counted are:

- Disposition table: A participant who, on the "Completion at End of Active Phase" form question "What was the participant's status?" has "Adverse Event" checked.
- Safety overview table: A participant who has either on the "Completion at End of Active Phase" form, question" What was the participant's status?" has "Adverse Event" checked or lists a solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.
- System Organ Class (SOC)/Preferred Term (PT) table: A solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.

4.2.3 Immunogenicity

4.2.3.1 Computed Values for Analysis

For the derivation of immunogenicity endpoints, all values strictly under the lower limit of quantification (LLOQ) will be treated as LLOQ/2, and all values above or equal to the upper limit of quantification (ULOQ) will be treated as ULOQ.

For the analysis of the results of HAI assay which is performed in duplicate, the individual geometric mean (GM) of both values will be computed for each blood sample and each strain, after managing extreme values as described above. The computed value is then considered the titer for that particular blood sample.

Unique records will be recorded for SN titration.

4.2.3.2 Fold-rise

For the HAI immune response, the derived endpoint fold-rise is driven by both baseline (D01) and post-baseline (D29) computed values as described in Section 4.2.3.1 and is computed as individual ratio:

- 28 days after vaccination divided by D01.

Note: if pre-vaccination (D01) or post-vaccination (D29) values is missing, the fold-rise is missing.

4.2.3.3 Seroconversion

For HAI assay, SC is defined as a binary indicator. If a pre-vaccination (D01) titer < 10 (1/dil): post-vaccination titer ≥ 40 (1/dil) on 28 days after vaccination (D29), or ≥ 4-fold-rise for participants with a pre-vaccination titer ≥ 10 (1/dil), the derived SC indicator will be "Yes", otherwise will be "No".

Note: if pre-vaccination (D01) or post-vaccination (D29) value is missing, the SC is missing.

4.2.4 Derived Other Variables

4.2.4.1 Age for Demographics

The age of a participant in the study will be the calendar age in years at the time of inclusion, as collected in the eCRF.

The study group will be determined by the investigator:

- If the participant is between the age of 9 and 17 years at the time of inclusion; the participant will be assigned to study age group 9-17 years , otherwise ;
- If the participant is between the age of 18 and 49 years at the time of inclusion; the participant will be assigned to study age group 18-49 years .

4.2.4.2 Duration of a Participant in the Trial

The duration of a participant in the trial, including the D181 safety follow-up, is computed as follows:

- Maximum (visit dates, termination date, safety follow-up date) – V01 date + 1.

The duration of a subject in the active phase of the trial is computed as follows:

- Maximum (V02 dates, termination date) – V01 date + 1.

4.2.4.3 Duration of the Study

The duration of the study is computed in days as follows:

- Maximum (Visit dates, Termination date, safety follow-up date) – minimum (V01 date) + 1

The duration of the active phase of the study is computed in days as follows:

- Maximum (latest date of V02, latest date of termination during the active phase) − minimum (V01 date) + 1,

The duration of the D181 safety follow-up phase of the study is computed in days as follows:

- Maximum (date of D181 safety follow-up) − minimum (V02 dates, termination dates during the active phase) + 1.

5 Changes in the Conduct of the Trial or Planned Analyses

All analyses planned to be conducted after the interim analysis defined in section 3.5 are strictly descriptive.

If the study Team decides not to conduct this interim analysis as defined in section 3.5 and proceed with the completion of the collection of all safety (6 month-follow-up) data through the end of the study; no amendment of this SAP will be written regarding the non-conduct of this interim analysis (section 3.5).

This decision by the study Team would not affect the primary objective of this study and would not require an amendment of characteristics of any of the analyses defined and planned in this study, such as an adjustment of type I and type II errors.

Discarding the interim analysis at D29 from this study, will be reported in only the protocol amendment, and no amendment of this SAP will be written regarding the non-conduct of this interim analysis.

Additionally, blood visit window is increased at statistical analysis level to address operational constraints whilst maintaining clinical relevance of the immunology read-outs.

Moreover, the recruitment of children and adolescents from 9 to 17 years was revealed harder than the recruitment of adults from 18 to 49 years. Hence, the last adult was enrolled on January, 5th 2023, whereas last participant from 9 to 17 years was enrolled on April, 28th 2023.

To assess the impact of this delay in recruitment of participants from both age groups, a complementary analysis will be performed by describing the main immunogenicity endpoints in the period where participants from both age groups were enrolled (i.e., with all participants with D01 date until January, 5th 2023).

6 Supporting Documentation

6.1 Appendix 1 List of Abbreviations

AE	Adverse Events
AESI	Adverse events of special interest
AR	Adverse reactions
CRF	Case report form
DMC	Data Monitoring Committee
ESDR	Early Safety Data Review
FAS	Full analysis set
GCI	Global Clinical Immunology
GCP	Good Clinical Practice
GM	Geometric mean
GMT	Geometric mean of titer
GMC	Geometric mean of concentration
HAI	Hemagglutination Inhibition
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
LLOQ	Lower level of quantitation
MAAE	Medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not applicable
NC	Not computed
PPAS	Per-protocol analysis set
PT	Preferred term
RCDC	Reverse cumulative distribution curve
SAE	Serious adverse events
SafAS	Safety analysis set
SAP	Statistical analysis plan
SC	Seroconversion
SN	Seroneutralization
SOC	System organ class

PT	Preferred term
TLF	Tables, listings and figures
ULOQ	Upper level of quantitation

7 References

1. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-72.

2. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med. 1998;17(8):873-90.