Janssen Vaccines & Prevention B.V.*

Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled, First-in-human Phase 1/2a Study to Evaluate Safety, Reactogenicity and Immunogenicity of a Universal Influenza (Uniflu) Vaccine with INFLUENZA G1 mHA in Healthy Adults

Protocol VAC21148FLZ1001; Phase 1/2a

VAC21148 (Uniflu Vaccine)

* Janssen Vaccines & Prevention B.V. is a Janssen pharmaceutical company of Johnson & Johnson and is hereafter referred to as the sponsor of the study. The sponsor is identified on the Contact Information page that accompanies the protocol.

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Compliance: The study described in this report was performed according to the principles of Good Clinical Practice (GCP).

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VERSION HISTORY

Table 1 – SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1.0	30-Nov-2023	Not Applicable	Initial release
2.0	06-Sep-2024	Section 5.3.3: Updated the time to onset and duration of solicited AEs to present this information for all solicited AEs, not just the most frequent solicited AEs.	Due to low sample size, all events will be included in this table.
		Section 5.7.1.5.1: Updated table 5: full-length HA breadth panel to include Group 1 excluding H1 and Breadth coverage columns	Table 5 updated to clarify which antigens are included in the extended breadth score analysis and in the analysis of the phylogenetic distance based weighting method.
		Section 5.7.1.5.1: Add phylogenetic distance based weighting method for breadth panel summary. Added table 6: phylogenetic distance matrix. Added text to describe the analysis of extended breadth score, breadth coverage and extended breadth coverage. Described that the nasal antibody response will be performed on the same subset of subjects that are used for the analysis of the extended breadth ELISA panel. Also included information on nasal swab type.	This method combines the genetic distances between influenza HA antigens with the strength of the human sera to bind to these antigens. Table 6 is added to document the distance matrix used in the analysis. New additional analysis of the antigens included in the breadth panel, to account for all antigens of interest. Clarification for the analysis of the nasal antibody response.
		Section 5.5: Corrected the number of items included in the Flu-iiQ impact categories	Correction of typo in the number of items included in the Flu-iiQ impact categories.

1. INTRODUCTION

This is the statistical analysis plan (SAP) applicable for the VAC21148FLZ1001 trial and specifies the statistical methods for the planned analyses of safety, reactogenicity, and immunogenicity data collected in the study. The SAP (version 2.0) is based on Clinical Trial Protocol (CTP) VAC21148FLZ1001 Amendment 2. Titles, mock-ups, and programming instructions for all statistical outputs (tables, figures, and listings) will be provided in a separate document, the Data Presentation Specifications (DPS). This SAP will be finalized prior to the database lock for the final analysis.

1.1. Objectives and Endpoints

Refer to Section 3 of the CTP for a list of objectives and endpoints.

1.2. Study Design

Refer to Section 4.1 of the CTP for details on the study design, and section 6.3 of the CTP for details on randomization and procedures for maintaining the blind.

2. STATISTICAL HYPOTHESES

No formal statistical hypothesis on safety is to be tested. The study will evaluate whether INFLUENZA G1 mHA, with or without $Al(OH)_3$ adjuvant, is safe, well tolerated, and immunogenic in healthy adults aged ≥18 to ≤45 years.

Safety data will be analyzed descriptively per vaccine group (placebo from Cohort 1 and 2 will be combined), with special attention to SAEs, Grade 3/4 AEs, and AEs leading to discontinuation of study vaccine.

No formal hypothesis on immunogenicity will be tested. Immunogenicity data will be analyzed descriptively per study group. Descriptive statistics (geometric mean and 95% CI, or median and quartile range [Q1-Q3], as appropriate) will be calculated for continuous immunological parameters at all time points and for changes from baseline.

3. SAMPLE SIZE DETERMINATION

Refer to Section 9.2 of the CTP for details.

4. POPULATIONS (ANALYSIS SETS) FOR ANALYSIS

Population	Description
Full Analysis Set (FAS)	The full analysis set will include all participants with at least one vaccination documented. All safety and participant information analyses will be based on the FAS.

Population	Description
Per-protocol Immunogenicity (PPI)	The PPI population will include all randomized and vaccinated participants for whom immunogenicity data are available excluding samples taken on or after the date when a participant experiences a major protocol deviation expected to impact the immunogenicity outcomes. In addition, samples obtained after missed doses or samples obtained after natural infection (if applicable) will be excluded from the analysis. • For participants who experience a natural influenza infection (based on RT-PCR, or other sources), samples collected within 7 days prior to and after the natural infection (e.g. start of reporting of ILI symptoms) will not be considered in the assessment of the immunogenicity of the selected regimen. • If a participant misses one or more active dose(s) of the selected regimen but continues the planned visit schedule, samples after the missed active dose(s) will not be considered. The analysis of all secondary and exploratory immunogenicity endpoints will be based on the PPI set. A sensitivity analysis may be performed to assess the impact of suspected sub-clinical influenza infection on immunogenicity. Suspected sub-clinical influenza infection will be defined at a given time point if a participant has a fold-increase from baseline for the full-length H5 ELISA four times greater than the fold-increase from baseline for the H5 VNA. Samples collected after suspected sub-clinical influenza infection will be excluded from this analysis. Depending on the number of samples excluded, an exploratory analysis might be performed, including the excluded samples. To visualize excluded samples, participant profiles from several assays might be repeated, indicating the excluded samples.

Vaccine assignment will follow the as-treated principle (participant will be presented according to received vaccine group).

5. STATISTICAL ANALYSES

5.1. General Considerations

All data will be analyzed descriptively per vaccine group. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, mean, standard deviation (SD), median, quartiles (Q1 and Q3), minimum, and maximum. The minimum and maximum will be reported to the same number of decimal places, the mean, median, lower, and upper quartile will be reported to one more decimal place and the SD will be reported to two more decimal places than the raw data recorded in the database. Frequencies and percentages (one decimal place) will be generated for categorical variables. Percentages will not be presented for zero counts.

5.1.1. Study Phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to the vaccination on Day 1.

The safety analysis will present all results by phase. Immunogenicity results will be presented per scheduled time point as appropriate. Listings will be shown per phase and time point.

Study day or relative day is defined as follows:

- Study Day = visit date – date of Day 1 + 1; if visit date >= date of Day 1 (date of first vaccination).
- Study Day = visit date – date of Day 1; if visit date < date of Day 1 (date of first vaccination).

5.1.2. Phase Definitions

The phases in the study will be constructed as follows:

Table 1: Phase Definitions

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form[a]	One minute prior to start of Post-dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to Post-dose 2
Follow-up 1	3	Follow-up 1		One minute after post-Dose 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim c) One minute prior to Post-Dose 2
Regimen	2	Post-dose 2	2	Date and time of second vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days)
Follow-up 2	4	Follow-up 2		One minute after Post-dose 2 period end	Minimum of: a) 23:59 at the date of last contact b) 23:59 at the date of database cut-off date in case of interim

[a] In case an earlier date is available (e.g., for lab or vital signs), then use the very first date to include all data.
Note: participants who did not receive a second vaccination will not have a Post-dose 2 or Follow-up 2 period.

The periods/phases will be used primarily for allocation of adverse events to various time periods during the study. Adverse events will be summarized for each of the active periods, 'Post-dose 1' and 'Post-dose 2', as well as Post-dose 1 and Post-dose 2 Combined, which is referred to as

'Regimen'. Additionally, for SAEs and AEs leading to discontinuation of study vaccine tables, an 'Entire study' period will be defined. 'Entire study' covers the time window from vaccination 1 up to and including the end of the study for each participant.

5.1.3. Visit Windows

For the immunogenicity analysis, assessments will be allocated to an analysis visit based on the planned visit as captured in the CRF. Visits that are out of the immunogenicity analysis (broad) visit windows (see Table 2) will not be included in the per-protocol immunogenicity analysis. However, they may be included in sensitivity analyses.

Table 2: Visit Windows for Immunogenicity Analysis

CTP Visit Day	Reference day	Target day (from reference day)	CTP visit (narrow)		Immunogenicity analysis (broad)	
			Window	Relative day	Window	Relative day
Day 1	Vac 1	Day 1	[0; 0]	[1;1]	[0; 0]	[1;1]
Day 8	Vac 1	Day 8	[0;2]	[8;10]	[-2;3]	[6;11]
Day 29	Vac 1	Day 29	[-3;3]	[26;32]	[-7;10]	[22;39]
Day 57	Vac 1	Day 57	[-3;3]	[54;60]	[-10;10]	[47;67]
Day 64	Vac 2	Day 8	[0;2]	[8;10]	[-6;7]	[2;15]
Day 85	Vac 2	Day 29	[-3;3]	[26;32]	[-14;14]	[15;43]
Day 238	Vac 2	6 months	[-14;14]	[168;196]	[-21;21]	[161;203]
Day 365	Vac 1	12 months	[-30;30]	[335;395]	[-84; ∞]	[≥281]

Time windows may be redefined prior to unblinding if the number of samples excluded as per the definitions above are too numerous.

5.2. Participant Dispositions

Participant information will be shown for the full analysis set.

The number of participants in the following disposition categories will be summarized throughout the study by intervention group and overall:

- participants screened
- participants randomized
- participants vaccinated and not randomized
- participants randomized and not vaccinated
- participants in the Full Analysis Set (FAS)
- participants in the Per-protocol Immunogenicity (PPI)
- participants who discontinued study
- participants who discontinued vaccination
- reasons for termination

Also, the number of participants and percentage per phase will be tabulated. The number of missed vaccinations will be tabulated.

5.2.1. Extent of Exposure

The number and percentage of participants who receive study vaccination, as well as the number of days between the 1st and 2nd vaccination, will be summarized by intervention group and overall. The number and percentage of participants by visit (Day 1, Day 8, Day 29, Day 57, etc.) will similarly be summarized.

5.3. Primary Endpoint(s) Analysis

5.3.1. Definition of Endpoint(s)

Section 3 of the CTP provides AE definitions associated with primary safety endpoints. The primary endpoints to assess safety are:

- Occurrence, severity, duration, and relationship to study vaccine of solicited local and systemic AEs for 7 days after each vaccination.
- Occurrence, severity, duration, and relationship to study vaccine of unsolicited AEs for 28 days after each vaccination.
- Occurrence and relationship to study vaccine of SAEs from first vaccination to the end of the study.

5.3.2. Estimand

Not applicable.

5.3.3. Analysis Methods

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively per intervention group and overall for the FAS. The number and percentage of participants with at least one particular AE (solicited/unsolicited) will be tabulated. Only treatment emergent (with onset or worsening after first vaccination) AEs will be presented in the tables.

Solicited AEs will be summarized by class (local or systemic) and preferred term. Unsolicited AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class and Preferred Term.

For solicited AEs, the following tables will be provided:

- summary,
- by worst severity grade,
- at least grade 3,
- related (systemic only),
- at least grade 3 related,

- time to onset (in days) from the start of each vaccination period and duration (in days), and
- body temperature.

Note: Duration is defined as number of days from the start of the event until resolution of the event. The time to first onset is defined as (date of first onset – reference date + 1). The reference dates are the start dates of all prior vaccination periods (i.e., the prior vaccination dates for dose 1 or dose 2).

For unsolicited AEs, the following tables will be provided:

- summary table (including SAEs, fatal outcome, and discontinuation),
- all events,
- at least grade 3,
- related,
- at least grade 3 related,
- SAEs
- time to onset (in days) from the start of each vaccination period and duration (in days).

5.4. Secondary Endpoint(s) Analysis

5.4.1. Key Secondary Endpoint

5.4.1.1. Definition of Endpoint

The secondary endpoint to evaluate immunogenicity is the magnitude of antibodies binding to the stem or the full-length HA protein as measured by enzyme-linked immunosorbent assay (ELISA).

5.4.1.2. Estimand(s)

Not applicable.

5.4.1.3. Analysis Methods

The analysis methods are described in Section 5.7.1.

5.5. Tertiary/Exploratory Endpoint(s) Analysis

Exploratory endpoints relating to immunogenicity are described in Section 5.7.1 and corresponding subsections.

Exploratory endpoints relating to evaluation of the virology objective include:

- Signs and symptoms of influenza-like infection (ILI)

Confirmation of infection by reverse transcriptase polymerase chain reaction (RT-PCR). Refer to section CTP 8.1.1. ILI Procedures for data collection details.

Two dimensions (symptom intensity and impact) are collected using 5 scales (3 to 7 items in each scale) from the FLU-iiQ questionnaire:

1. Symptom intensity
    - respiratory symptoms (3 items)
    - systemic symptoms (7 items)
2. Impact
    - daily activities (6 items)
    - other people (5 items)
    - emotions (4 items)

The items within scales are coded (0=None, 1=Mild, 2=Moderate, 3=Severe), then scales are averaged [scale score divided by the number of items], thus each scale ranges from 0 to 3.

Total score (average of all 5 scales), total symptom intensity score (average of respiratory symptoms and systemic symptoms score) and total impact score (average of daily activities, other people, and emotions scores) will be calculated. If more than 50% of data from one scale is missing, then the value for that scale for that individual is coded as missing.

Descriptive statistics by intervention group might be provided for each scale of FLU-iiQ evaluating signs and symptoms for subjects developing ILI during the influenza season, if more than 10 participants report symptoms. Otherwise, listing of FLU-iiQ responses will be provided.

The number and percentage of participants who have signs and symptoms of ILI and confirmation of influenza infection by RT-PCR or response to ILI by serology will be summarized.

5.6. Other Safety Analyses

Safety analyses will be performed on the FAS. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), 95% CI for the mean (if applicable, large studies), standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum, and maximum. Frequencies and percentages (one decimal place) will be generated for categorical variables. No formal comparisons between groups will be provided.

Two types of safety tables will be shown. In the by regimen layout, safety data will be analyzed by study intervention regimens as designed per protocol. In case of multiple vaccinations, data will be presented by phase as well as over the entire regimen. The denominator for the percentages is the number of participants in the considered population and phase for a certain regimen (incidence per 100 participants/phase). In the second, by vaccine layout, only limited safety data will be presented by the different vaccines used in the study but by considering every vaccination administered as a separate safety episode. The denominator for the percentages is the number of vaccinations administered of a certain vaccine (rate per 100 vaccinations of a particular vaccine). DPS will define which tables will be provided by vaccine layout.

5.6.1. Adverse Events

5.6.1.1. Definitions

Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined local events (pain/tenderness, erythema, and swelling at the injection site) and systemic events (fatigue, headache, nausea, myalgia, and fever) for which the participant is specifically questioned, and which are noted by participants in their reactogenicity diary. Unsolicited AEs are all AEs for which the participant is not specifically questioned.

Solicited AEs shown in the tables are extracted from the investigator assessment pages (CE) of the CRF. For unsolicited AEs, only the AEs within the 28-day period following each vaccination will be presented in the safety tables. SAEs will be captured and tabulated in the outputs covering the whole study period. All other collected unsolicited adverse events will be presented through listings.

The verbatim terms used in the eCRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

Solicited administration site symptoms will be considered as related to the study vaccine.

The severity of the AEs will be classified as grade 1 to 4. Solicited events that are graded less than grade 1, are not considered as AE. In case no grades are available, the grading of the solicited events will occur according to the grading list in Appendix 6.

5.6.1.2. Analysis of Adverse Events

Analysis of adverse events is described in Section 5.3.3.

Unsolicited AEs may also be presented for Standardized MedDRA Queries (SMQs) to facilitate review of certain classes of unsolicited AEs.

5.6.1.3. Phase Allocation of Adverse Events

Step 1: Allocation of events to the periods:

Solicited events are always allocated to the respective Post-dose period.

Unsolicited adverse events are allocated to periods based on their start date/time. If the start date/time of an event falls between (or on) the start and stop date/time of a period, the AE is attributed to that period (treatment-emergent principle).

- In case of partial start or stop dates (i.e. time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.

- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the study. In case of a completely missing start date, the event is

allocated to the first active treatment phase (Post-dose 1 period), except if the end date of the AE falls before the start of the first active treatment phase (Post-dose 1 period).

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same participant with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1. If overlapping/consecutive events start in one of the following phases/periods - Screening or Follow-up (defined as non-active periods) - followed by an AE in – a Post-dose period (defined as active period) - they are allocated to their respective phases/periods and are considered as separate events.
2. In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.
3. In case overlapping/consecutive events start in both an active period followed by one or more consecutive non-active periods, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, treatment period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.
4. In case an active period is followed by another active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period and are considered as separate AEs. In case overlapping/consecutive events start in non-consecutive periods (regardless of active or non-active), they are allocated to their respective period and are considered as separate AEs.
5. In case a non-active period is followed by another non-active period, and the overlapping/consecutive events start in both periods, they are allocated to the first period and they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

Remarks:

1. Events can only be combined into one and the same AE if their start and stop dates are known.
2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.
3. Time is not considered when determining overlap of events.

5.6.1.4. Missing Data

Missing data will not be imputed. Participants who do not report an event will be considered as participants without an event. An AE with a missing severity or relationship will be considered as an AE reported, but will be considered as not reported for the severity or relationship. For example,

an AE with missing severity will be considered as an AE reported for the analysis of any grade but will be considered as not reported for the analysis of at least grade 3.

5.6.2. Laboratory, Vital Signs, and Physical Examination

For laboratory safety parameters and vital signs, only abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Protocol Appendix 5.

Vital signs including temperature, pulse/heart rate, respiratory rate, and blood pressure (systolic and diastolic) will be summarized over time, using descriptive statistics. Abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Protocol Appendix 5.

An abnormality (toxicity grade or abnormality based on normal ranges) will be considered as emerging in a particular period if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging. A shift from 'abnormally low' at baseline to 'abnormally high' post baseline (or vice versa) is also emerging. In case a laboratory test result is censored (no numeric value is available, but only a verbatim term) then a numeric value will be imputed by a value exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x; eg <3.45 is imputed with 3.44).

In case no toxicity grades are defined for a test, the abnormalities (above/below normal range) will be used. In determining toxicity grades, the following rules are applied:

- Worst grades/abnormalities are determined over the whole observational period for each trial period separately, including all post-baseline measurements of that period.
- The abnormalities 'abnormally low' and 'abnormally high' are considered equally important, i.e., if a participant has an abnormally low as well as an abnormally high value post-baseline, both abnormalities are shown in the tables. (This means that the sum of the percentages can be more than 100%)
- Note: as the grading scale for some parameters in the grading table has some gaps (zones where no toxicity grade definition exists), laboratory results falling in these zones will be allocated to the adjacent worst-case grade.
- If a lab value falls within the grading as specified in the grading table but also within the local lab normal limits, the value is considered as normal.

For the grades, no distinction will be made between test results of samples obtained under fasting and under non-fasting conditions: in case limits under fasting and non-fasting conditions differ, the limits of the conditions (fasting/non-fasting) of scheduled visits as planned in the CTP will always be used, also for samples obtained under a different condition (e.g., samples of withdrawal visits).

A listing of participants with fever according to the FDA grading table will also be provided. In addition, temperature measurements (whether obtained from the diary or from on-site assessments) will be allocated to predefined temperature intervals (from 37.5° C until 40°C, in steps of half degree increments; eg <37.5, 37.5-<38, 38-<38.5, … >40), and the worst temperature reported over the first 7 first days after a vaccination (Day 1 to Day 8, from any of the diary or the on-site assessments) will be tabulated by intervals.

5.7. Other Analyses

5.7.1. Immunogenicity

The analysis of immunogenicity will use the PPI set. A sensitivity analysis excluding additional samples after suspected sub-clinical influenza infection might be performed.

Data will be presented by the vaccine group (placebo from Cohort 1 and 2 will be combined) and by the scheduled time point. For the PPI analysis, samples taken outside of the allowed window will be excluded from the tables and graphs (but will be included in the listings and clearly marked as results not included in the PPI analyses).

5.7.1.1. Parameters

Immunogenicity assessments may include, but are not limited to, the humoral and cellular immunogenicity assays (as available and applicable) summarized in Table 3.

Table 3: Summary of Immunogenicity Assays

Endpoint	Assay
Secondary endpoints	
Quantification of antibody binding to HA stem or full-length HA	ELISA
Exploratory endpoints	
Neutralization	Virus neutralization assay
Fc-mediated functionality	Human FcγRIIIa reporter assay
Breadth of the antibody response within Group 1 or beyond	ELISA using full-length HAs indicative of clade or group coverage
HA stem- or epitope-specificity	ELISAs using HA stem protein or competition ELISAs using bnAbs or HA proteins
Functional breadth of the antibody response within Group 1 or beyond	Virus neutralization assays or serum transfer into animal challenge models
Functional characterization of the antibody response	Assays assessing Fc-mediated functions (eg, antibody-dependent cytotoxicity assay, or antibody-dependent cellular phagocytosis assay), HA conformational change assays, or other assays
Molecular characterization of the antibody response	Assays assessing the epitope-specificity, glycosylation status, or other characteristics of the antibody (eg, systems serology arrays)
Innate immune responses	Transcript- or cytokine profiling
Gene expression profiles	RNA sequencing or other transcriptional profiling methods, including, but not limited to TCR or BCR repertoire analysis
Nasal antibody response	IgA and/or IgG ELISA in nasal secretions
Hemagglutination inhibition	HAI assays

BCR = B cell receptor; bnAbs = broadly neutralizing antibodies; ELISA = enzyme-linked immunosorbent assay; Fc = fragment crystallizable; HA = hemagglutinin; HAI = hemagglutination inhibition; Ig = immunoglobulin; RNA = ribonucleic acid; TCR = T cell receptor.

Table 4 Presents the core assay package for this study.

Table 4: Core assay package for VAC21148FLZ1001

Parameter	Assay	Antigen
Stem-binding Ab (core assays)	H5 IgG ELISA and using trimeric full-length HA of a heterosubtypic Group 1 strain	H5 A/Vietnam/1203/2004 monobasic cleavage site, uncleaved (UFV190837)
	Mini-HA ELISA	Mini-HA protein with H1 A/Cal/09 backbone
Functionality	Neutralization by H5 Plaque Reduction Neutralization Test (PRNT) Influenza-virus HA matching core ELISA	H5N1 NIBRG-14 reassortant of A/Vietnam/1194/2004(H5N1) and A/PR/8/34(H5N1), polybasic cleavage site excised
	Fc-mediated effector function by H5 Human FcγRIIIa reporter assay (Promega) HA matching core ELISA	H5 A/Vietnam/1203/2004, monobasic cleavage site, uncleaved, membrane bound (UFV191437)
Breadth panel	IgG ELISAs using additional trimeric full-length HAs antigens	See breadth-panel Table 5
Locality	IgA and/or IgG ELISA in nasal secretions	H5 A/Vietnam/1203/2004 monobasic cleavage site, uncleaved (UFV190837)
Durability	H5 IgG ELISA and using trimeric full-length HA of a heterosubtypic Group 1 strain	H5 A/Vietnam/1203/2004 monobasic cleavage site, uncleaved (UFV190837)

5.7.1.2. Handling of Missing and/or Unquantifiable Immune Response Data

Missing immune response data will not be imputed.

Values below the lower limit of quantification (LLOQ) will be imputed based on the type of analysis:

- For the calculation of the geometric mean titer, values below LLOQ will be imputed to LLOQ/2.
- For the calculation of the geometric mean of the increase from baseline and calculation of the Breadth Coverage, values below LLOQ will be imputed to LLOQ.

Data above the upper limit of quantification (ULOQ) will be imputed with the ULOQ.

The LLOQ or limit of detection [LOD], as applicable, and ULOQ values per assay are available in the database.

5.7.1.3. Handling of Changes in Assay Status throughout the Study Conduct

In case of changes in assay status, for example in case the assay range is extended or when going from “qualified” to “validated”, the LLOQ and ULOQ are likely to change as well. Should this happen, then the SDTM database will contain records pertaining to the assay in the qualified status and records pertaining to the validated status, and the LLOQ and ULOQ values will also differ.

5.7.1.4. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

5.7.1.5. Immunogenicity

5.7.1.5.1. Humoral assays

For ELISAs (H5-ELISA and mini-HA ELISA) and **VNA** the following results will be calculated: N, geometric mean§ and corresponding 95% CI of the actual values, and fold increases from baseline will be tabulated and graphically presented. *§calculate the mean and corresponding 95% CI of log_{10} transformed values, back-transform this mean [i.e., 10^{mean}] and CI [i.e., 10^{CI}].*

A sample is considered positive if the value is above the assay LLOQ.

A response will be defined as:

- if sample interpretation is negative at baseline and there is >4-fold increase from LLOQ (on the original scale), or,
- if sample interpretation is positive at baseline and there is a >4-fold increase from baseline (on the original scale).

Tables will show the sample positivity, responders, and the percentage of participants with a >2-fold increase.

Geometric mean ratios (GMR) § between active groups and placebo group (pooled across cohort 1 and 2), with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA), using log-transformed post-baseline value as dependent variable and study group as independent variable. As a sensitivity analysis, the ANOVA may also be performed adjusting for the respective baseline titers, by including log-transformed baseline titer as additional covariate. *§by back-transform LSmean and corresponding 95% CI from ANOVA [i.e., 10^{LSmean}] and CI [i.e., 10^{CI}].*

Actual values and fold changes from baseline are tabulated and shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay.

In the graphs, original values will be displayed on the log_{10} scale. The actual values will be shown, even if they are below the LLOQ. The LLOQ cut-off will be visualized in the graph per assay. Participant profiles of the actual values over time will also be graphically presented. A correlation plot of H5 ELISA versus mini-HA ELISA will be provided for the most important time points (at minimum, Baseline, Day 29, and fold increase from Baseline at Day 29).

A correlation plot with the H5-ELISA versus. VNA, H5-ELISA vs. Human FcγRIIIa reporter assay, VNA vs. Human FcγRIIIa reporter, H5-ELISA vs. IgA and/or IgG ELISA in nasal secretions will be provided for the most important time points (at minimum, Baseline, Day 29 and fold increase from Baseline at Day 29). In these scatterplots the actual values will be shown, even if they are below the LLOQ. The LLOQ cut-off will be visualized in the graph per assay.

Additional graphs might be considered based on the review of the first data results, potentially assessing the role of pre-existing immunity on the vaccine induced immune response.

Additional graphs might be considered for participants who developed ILI symptoms or have RT-PCR influenza infection.

Human FcγRIIIa reporter assay data will be presented in a similar way to core assays as described above.

Breadth of the antibody response within Group 1 will be assessed by IgG ELISAs using additional trimeric full-length HA breadth panel as presented in Table 5 below:

Table 5: full-length HA breadth panel

HA	Year	Strain	Rationale	Q**	Narrow H1 score	Broad Group 1 score	Group 1 score excl. H1	Breadth Covera ge
H5	2005	A/Vietnam/1203/2004 (UFV190837)	Core assay	F		Y	Y	Y
H5*	2023	A/Cambodia/NPH230032/2023 (UFV235130)	Pandemic recent avian strain	P		Y	Y	Y
mH1^	2009	A/California/07/2009 (UFV180702)	H1 subtype, vaccine backbone	F				
mH1*	1999	A/New Caledonia/20/1999 (UFV190137)	H1 subtype, remote, benchmark VRC	P	Y	Y		
H1	2019	A/Victoria/2570/2019 (UFV221619)	H1 subtype	F	Y	Y		Y
H1	2009	A/California/07/2009 (UFV181009)	H1 subtype, vaccine backbone	F	Y	Y		Y
H1	1999	A/New Caledonia/20/1999 (UFV181089)	H1 subtype, remote, benchmark VRC	P	Y	Y		Y
H1	1918	A/South Carolina/1/1918 (UFV181084)	H1 subtype, oldest, pandemic	P	Y	Y		Y
H2	2005	A/Env/MPU3156/2005 (UFV181154)	Pandemic	P		Y	Y	Y
H2*	2020	A/avian/Japan/KU-d3c/2020 (UFV235127)	Pandemic recent avian strain	P		Y	Y	Y
H11	2009	A/Env/MPK655/2009 (UFV190050)	H11 clade, representative	F		Y	Y	Y
H17	2010	A/bat/Guatemala/060/2010-(UFV190 401)	Bat strain, stable molecule	P		Y	Y	Y
H9	1999	A/Hong Kong/1073/1999 (UFV181156)	H9 clade, benchmark PATH	F		Y	Y	Y
H9*	2021	A/Cambodia/21020301/2021 (UFV235131)	Pandemic recent avian strain	P		Y	Y	Y
H7^	2013	H7N9 A/Anhui/1/2013 (UFV180438)	G2, pandemic, low pressure	E				
B^	2017	B/Iowa/06/2017(UFV235091)	B, dominant Victoria lineage	E				

* Included in extended breadth score only (are available only for subset of participants).
** Q = Qualification status: P = partially qualified, F = fully qualified, E = exploratory.
^ mH1 (A/California/07/2009), H7 (H7N9 A/Anhui/1/2013), and B (B/Iowa/06/2017) will be reported separately and are not included in any breadth score.
Y = included in corresponding breadth score or breadth coverage calculation.

Each IgG ELISA from the breadth panel data will be presented in a similar way to the core ELISA assays as described above.

Breadth Score

Three types of breadth scores will be calculated based on breadth panel assays, listed in Table 5 using Maximum Diversity Weighting method (Z. He, Y. Fong, Stat Med. 2019) (R package: "mdw"):

- Narrow H1 score (H1 strains as identified in Table 5):
  - Although the vaccine backbone mini-HA ELISA does assess an H1 HA, this ELISA may also measure neo-epitope immune responses against design elements of the protein, which could potentially result in an inflated mini-HA ELISA response in FLZ1001. Therefore, the vaccine backbone mini-HA ELISA (A/California/07/2009) results will be excluded from the H1 Breadth Score.
- Broad Group 1 score (Group 1 strains as identified in Table 5):
  - The vaccine backbone mini-HA ELISA (A/California/07/2009) results will be excluded from the Group 1 Breadth Score (same reason as for Narrow H1 score).
  - The H7 and B ELISAs will also be excluded from the Group 1 Breadth Score as these are Group 2 and Flu B antigens, respectively, and do not represent Group 1 HAs.
- Group 1 score excluding H1 (Group 1 strains as identified in Table 5):
  - All H1 (including the mini-HA ELISA) results will be excluded from the Group 1 score excluding H1.
  - The H7 and B ELISAs will also be excluded from the Group 1 score excluding H1 as these are Group 2 and Flu B antigens, respectively, and do not represent Group 1 HAs.

The Breadth score will be calculated for each participant only when all ELISAs used in the calculation are non-missing. For each ELISA included in the breadth score calculation, values below LLOQ will be imputed to LLOQ/2, and values above the upper limit of quantification (ULOQ) will be imputed with the ULOQ. Calculation of the breadth score is done separately at each analysis.

The following results will be calculated: N, geometric mean[§] and corresponding 95% CI, interquartile range, min, max of the actual values, and fold increases from baseline will be tabulated and graphically presented. [§]*calculate the mean and corresponding 95%CI of* log_{10} *transformed values, back-transform this mean [i.e.,* 10^{mean}*] and CI [i.e.,* 10^{CI}*].*

Geometric mean ratios (GMR)[§] between active groups and placebo group (pooled across cohort 1 and 2), with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA), using log-transformed post-baseline value as a dependent variable and study group as independent variable. As a sensitivity analysis, the ANOVA may also be performed adjusting for the respective baseline value, by including log-transformed baseline value as an additional covariate. [§]*by back-transform LSmean and corresponding 95%CI from ANOVA [i.e.,* 10^{LSmean}*] and CI [i.e.,* 10^{CI}*].*

Fold changes from baseline will be shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay.

Extended Breadth Score

The analysis of extended breadth score will be performed on a subset of participants from the PPI analysis set who have samples for analysis at both Day 1 and Day 29.

A random selection of about 75 participants in total from three FLZ1001 study groups and 76 participants from SF-SC cohort from VAC31518COV3005 study (see section 5.7.1.6.1) will be made. For FLZ1001, all participants meeting criteria from the INFLUENZA G1 mHA CCI - INFLUENZA G1 mHA CCI group will be included in the subset. A random sample of 30 participants from the pooled group of INFLUENZA G1 mHA CCI - INFLUENZA G1 mHA CCI and INFLUENZA G1 mHA CCI – placebo groups and from the pooled group of INFLUENZA G1 mHA CCI $Al(OH)_3$ - INFLUENZA G1 mHA CCI $Al(OH)_3$ and INFLUENZA G1 mHA CCI $Al(OH)_3$ – placebo groups will be made respectively. Selecting 30 subjects will allow for a total of 25 subjects to be included in the subset from each of those two groups, where subjects with vials with low volume are excluded.

The same three types of breadth scores (Narrow H1 score, Broad Group 1 score, and Group 1 score excluding H1) will be calculated based on the extended breadth panel assays (as identified in Table 5) using Maximum Diversity Weighting method. The ELISAs that were included in the original breadth scores are also included in the extended breadth scores for selected subjects. The extended breadth scores will include additional ELISAs as detailed below:

- Narrow H1 score (H1 strains as identified in Table 5):
  - The same ELISAs that are included in the Narrow H1 score.
  - In addition, the mini-HA ELISA (A/New Caledonia/20/1999) is included.
- Broad Group 1 score (Group 1 strains as identified in Table 5):
  - The same ELISAs that are included in the Broad Group 1 score.
  - In addition, the following ELISAs are included: mini-HA (A/New Caledonia/20/1999); H5 (A/Cambodia/NPH230032/2023); H2 (A/avian/Japan/KU-d3c/2020); and H9 (A/Cambodia/21020301/2021).
- Group 1 score excluding H1 (Group 1 strains as identified in Table 5):
  - The same ELISAs that are included in the Group 1 score excluding H1.
  - In addition, the following ELISAs are included: H5 (A/Cambodia/NPH230032/2023); H2 (A/avian/Japan/KU-d3c/2020); and H9 (A/Cambodia/21020301/2021)

Analysis will be done in a similar way as described above for the Breadth score.

Breadth Coverage

Another breadth panel summary will be based on the phylogenetic distance based weighting method. The algorithm weighs the fraction of ELISA antigens that reach a given concentration with a distance factor, favoring ELISA antigen pairs with greater sequence distances, and therefore show broader coverage

Using the *Neighbor Joining method* and *Jukes-Cantor distance measure* and constructed in CLC Main Workbench 23.0.5 (QIAGEN Aarhus, Denmark), HA amino acid sequence alignment and

the phylogenetic tree will be generated. For each pair of IgG binding ELISAs as identified in Table 5, the phylogenetic distance factor was calculated (Table 6).

Table 6: Phylogenetic distance matrix

HA	Strain	H1_Cal	H1_Vic	H1_SC	H1_NC	H2_2005	H2_2020	H5_cam	H5_Vtnm	H11	H17	H9_HK	H9_Camb
H1	A/California/07/2009 (UFV181009)	0	0.05	0.15	0.24	0.45	0.43	0.49	0.47	0.63	0.71	0.74	0.73
H1	A/Victoria/2570/2019 (UFV221619)	0.05	0	0.18	0.25	0.45	0.43	0.5	0.47	0.62	0.69	0.72	0.71
H1	A/South Carolina/1/1918 (UFV181084)	0.15	0.18	0	0.16	0.39	0.37	0.46	0.43	0.61	0.67	0.64	0.69
H1	A/New Caledonia/20/1999 (UFV181089)	0.24	0.25	0.16	0	0.45	0.42	0.47	0.47	0.64	0.7	0.68	0.7
H2	A/Env/MPU3156/2005 (UFV181154)	0.45	0.45	0.39	0.45	0	0.03	0.3	0.28	0.62	0.65	0.66	0.7
H2*	A/avian/Japan/KU-d3c/2020 (UFV235127)	0.43	0.43	0.37	0.42	0.03	0	0.29	0.27	0.59	0.63	0.65	0.68
H5*	A/Cambodia/NPH230032/2023 (UFV235130)	0.49	0.5	0.46	0.47	0.3	0.29	0	0.1	0.61	0.69	0.73	0.78
H5	A/Vietnam/1203/2004 (UFV190837)	0.47	0.47	0.43	0.47	0.28	0.27	0.1	0	0.59	0.68	0.7	0.77
H11	A/Env/MPK655/2009 (UFV190050)	0.63	0.62	0.61	0.64	0.62	0.59	0.61	0.59	0	0.79	0.69	0.69
H17	A/bat/Guatemala/060/2010-(UFV19 0401)	0.71	0.69	0.67	0.7	0.65	0.63	0.69	0.68	0.79	0	0.79	0.77
H9	A/Hong Kong/1073/1999 (UFV181156)	0.74	0.72	0.64	0.68	0.66	0.65	0.73	0.7	0.69	0.79	0	0.15
H9*	A/Cambodia/21020301/2021 (UFV235131)	0.73	0.71	0.69	0.7	0.7	0.68	0.78	0.77	0.69	0.77	0.15	0

The Breadth coverage area under the curve (AUC) will be calculated for each participant only when all ELISAs used in the calculation are non-missing. For each ELISA included in the breadth coverage calculation, values below LLOQ will be imputed with the LLOQ, and values above the upper limit of quantification (ULOQ) will be imputed with the ULOQ. All ELISA values will be normalized to $\log_{10}$ [original value / LLOQ value] prior to calculation of the Breadth coverage.

Breadth coverage plots will be generated based on all relevant IgG binding ELISAs and their distance factors for each subject. To quantify the coverage of breadth of binding ELISAs, the area under the curve (AUC) will be calculated by using the trapezoidal rule. Breadth coverage plots and AUC calculations will be generated and performed using R package “tidyverse” and “ggprism”.

For the Breadth coverage AUC, the following results will be calculated: N, mean and corresponding 95% CI, interquartile range, min, max of the actual values, and mean increases from baseline will be tabulated and graphically presented. Reverse cumulative breadth coverage plots for each group will be presented based on individual breadth coverage. Least square means between active groups and the placebo group (pooled across cohort 1 and 2), with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA), using post-baseline value as a dependent variable and study group as independent variable. As a sensitivity analysis, the ANOVA may also be performed adjusting for the respective baseline value, by including baseline value as an additional covariate.

Extended Breadth Coverage

Same as for extended breadth scores, Extended Breadth Coverage was calculated using additional extended breadth panel. Results are presented in a similar way as described above for the Breadth coverage.

Nasal antibody response may be assessed using IgA and/or IgG ELISA in nasal secretions and presented in a similar way as described above for the core ELISAs and VNA. Nasal antibody response will be done on the same subject selection as for extended breadth ELISA panel for the FLZ1001 study only at Day 1 and Day 29. Two different types of nasal swab are used in the study, namely nylon flock and SAM. Attempt to normalize values reported by two different swab types will be made to be able to pool results in same summary table. If normalization will not be successful, the data will instead be summarized by swab type.

5.7.1.6. External control group from VAC31518COV3005 study

VAC31518COV3005 (COV3005) is a randomized, double-blind, phase 3 study to evaluate safety, reactogenicity, and immunogenicity of co-administration of Ad26.COV2.S and influenza vaccines in healthy adults 18 years of age and older. The study aims to assess the safety, reactogenicity, and immunogenicity of the Ad26.COV2.S vaccine co-administered with a quadrivalent standard-dose or high-dose seasonal influenza vaccine compared to administration of each vaccine separately to explore whether Ad26.COV2.S and the influenza vaccines can be administered concomitantly.

Without a seasonal flu vaccinated control group in FLZ1001, samples from COV3005 participants will be used to assess the performance of FLZ1001 assays and stem binding response upon seasonal influenza vaccination. Samples from COV3005 participants will be used as an external control group and referred to as a Seasonal Flu vaccinated reference Serum Collection ("SF-SC"). Those samples will run alongside FLZ1001 study samples in all assays. The COV3005 study includes the same target population as the FLZ1001 study, healthy adults aged ≥18 to ≤45 years, and also includes older adults, the main envisioned target population of the Uniflu vaccine.

Details on the purpose and random selection of samples are presented in Universal Influenza Vaccine Study plan in COV3005 (TV-REF-281637).

5.7.1.6.1. Populations (analysis sets) for COV3005 analysis

Broad SF-SC: At the first stage, 200 subjects who received the standard dose seasonal flu vaccination and did not receive Ad26.COV2.S vaccination (Broad SF-SC) were randomly selected to assess the performance of FLZ1001 assays on seasonal flu-vaccinated subjects (stratified by age group).

SF-SC: At the second stage, out of those 200 subjects a subset (SF-SC) will be selected to match as much as possible the population enrolled in FLZ1001. A direct comparison of FLZ1001 immunogenicity data will be made to the SF-SC cohort.

5.7.1.6.2. Humoral assays

Descriptive statistics and graphs on humoral immunogenicity data listed in Table 4 (where available) will be done similarly as described in section 5.7.1.5.1. for the COV3005 Broad SF-SC cohort (H5 ELISA, mini-HA ELISA, VNA, Human FcγRIIIa reporter assay, breadth ELISAs including three breadth scores, coverage score, and HAI assay) and for the SF-SC cohort.

For H5 ELISA, mini-HA ELISA, VNA, Human FcγRIIIa reporter assay, and three breadth scores, geometric mean ratios (GMR) [§] between active groups (FLZ1001 vs. SF-SC cohort), with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA), using log-transformed 28 days post-dose 1 immune parameter response as dependent variable and study group as independent variable. As a sensitivity analysis, the ANOVA may also be performed adjusting for the respective baseline titers, by including log-transformed baseline titer as an additional covariate. *[§]by back-transform LSmean and corresponding 95%CI from ANOVA [i.e., 10^{LSmean}] and CI [i.e., 10^{CI}].*

In addition, GMR plots over time, combining the regimens in one graph (without individual participant dots) will also be created.

For the breadth coverage, least square mean differences between active groups (FLZ1001 vs. SF-SC cohort), with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA), using 28 days post-dose 1 breadth coverage as dependent variable and study group as independent variable. As a sensitivity analysis, the ANOVA may also be performed adjusting for the respective baseline value, by including baseline breadth coverage as an additional covariate.

HAI assays (for each of the 4 influenza vaccine strains from COV3005 study).

For HAI assays data following results will be calculated for each of 4 influenza vaccine strains used in the study: N, geometric mean[§] and corresponding 95% CI of the actual values and fold increases from baseline will be tabulated and graphically presented. *[§]calculate the mean and corresponding 95% CI of log_2 transformed values, back-transform this mean [i.e., 2^{mean}] and CI [i.e., 2^{CI}].*

- Seroconversion is defined for each of the influenza vaccine strains at 28 days after the administration of a seasonal influenza vaccine:
  - HI titer ≥1:40 in participants with a pre-vaccination HI titer of <1:10, or
  - a ≥4-fold HI titer increase in participants with a pre-vaccination HI titer of ≥1:10
- Seroprotection is defined for each of the influenza vaccine strains as HI titer ≥1:40.

Tables will show the seroprotection, seroconversion, and fold increases.

Actual values and fold changes from baseline are tabulated and shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay.

In the graphs, original values will be displayed on the log2 scale. The actual values will be shown, even if they are below the LLOQ. The LLOQ cut-off will be visualized in the graph per assay.

A scatterplot with the age, mini-HA ELISA, and H5-ELISA versus HAI titers (per each 4 influenza vaccine strains), will be provided for the most important time points (at minimum, baseline, Day 29, and fold increase from baseline at Day 29).

A scatterplot with the HAI titers fold increase from baseline at Day 29 vs. baseline HAI titers will be provided.

5.7.2. Definition of Subgroups

- For FLZ1001 and the SF-SC cohort of COV3005, demographic data and immunogenicity data (selected tables and graphs on H5-ELISA, mini-HA ELISA, VNA, Human FcγRIIIa reporter assay, and Breadth of the antibody response) will be presented also by sex.
- For the Broad SF-SC cohort of COV3005, demographic data and immunogenicity data (selected tables and graphs on H5-ELISA, mini-HA ELISA, VNA, Human FcγRIIIa reporter assay, Breadth of the antibody response, and HAI titers) will be presented also by
    - sex
    - age group
        - 18-45 years
        - 46-64 years
        - >=65 years
    - imprinting groups (according to the birth year):
        - <1957 H1N1
        - 1957-1967 H2N2
        - 1968-1976 H3N2
        - 1977-2009 H3N2 or H1N1

5.8. Interim Analyses

There are no plans for interim analyses with an intent to stop the study early or to adapt the design or planned number of patients. However, there will be planned analyses at pre-specified timepoints as described in the study protocol (please refer to the CTP section 9.5 Planned Analyses).

DPS will identify which outputs will be produced for each analysis.

Based on safety and immunogenicity results, the sponsor's Vaccine Development Committee will decide which regimen will be taken forward. Additional factors such as data from other studies[*] or other assays, literature, epidemiology data, manufacturability, and ease of administration will be considered to select the regimen for the next clinical study.

[*]Additional analyses might be performed on immunogenicity data up to 29 days post-dose 1 from the external control group (COV3005 study). Two cohorts will be presented:

1. independent analysis will be performed on the Broad SF-SC cohort of COV3005 (200 subjects' cohort), followed by
2. selection of the SF-SC (matching ZLF1001 population) and analysis of the SF-SC cohort.

5.8.1. Data Monitoring Committee (DMC) or Other Review Board

An internal Data Review Committee (DRC), consisting of members that are not directly involved in the study conduct, data management, or statistical analysis, will be established for this study,

and will monitor data to ensure the safety and well-being of the participants enrolled. For further details please refer to:

- CTP section 10.3. Appendix 3: Regulatory, Ethical, and Study Oversight Considerations, **Data Review Committee** section.
- DRC charter (EDMS-ERI-199974976)
- DRC SAP (EDMS-ERI-206674654)

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 List of Abbreviations

ADaM	Analysis Data Model
AE	adverse event
ATC	anatomic and therapeutic class
BMI	body mass index
CI	confidence interval
CRF	case report form
CTP	clinical trial protocol
DMC	Data Monitoring Committee
DPS	data presentation specifications
DRC	Data Review Committee
ELISA	enzyme-linked immunosorbent assay
FAS	full analysis set
FDA	Food and Drug Administration
Flu-iiQ™	Influenza Intensity and Impact Questionnaire
GMC	Geometric Mean Concentration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titre
HAI	hemagglutination inhibition
ILI	Influenza-like illness
LLOQ	lower limit of quantification
LOD	Limit of detection
mini-HA	hemagglutinin stem-derived protein vaccine antigen
MedDRA	Medical Dictionary for Regulatory Activities
NA	not applicable
RT-PCR	reverse transcriptase polymerase chain reaction
PPI	per protocol immunogenicity analysis set
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SDTM	Study Data Tabulation Model
SF-SC	Seasonal Flu Vaccinated Reference Serum Collection
SMQ	standardized MedDRA query
ULOQ	upper limit of quantification
VNA	virus neutralizing antibody
WHO	World Health Organization

6.2. Appendix 2 Changes to Protocol-Planned Analyses

None to describe at the drafting of SAP version 1.0.

6.3. Appendix 3 Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.

Table 7 presents a list of the demographic variables that will be summarized by vaccine regimen and overall for the FAS.

Table 7: Demographic Variables

Continuous Variables:	**Summary Type**
Age (years)	Descriptive statistics (N, mean, standard deviation [SD], median, and range [minimum and maximum]).
Weight (kg)	
Height (cm)	
Body Mass Index (BMI) (kg/m^2)	
Categorical Variables	
Sex (male, female, undifferentiated, unknown)	Frequency distribution with the number and percentage of participants in each category.
Race[a] (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Not reported, Unknown)	
Ethnicity (Hispanic or Latino, not Hispanic or Latino)	

[a]If multiple race categories are indicated, the Race is recorded as 'Multiple'

6.4. Appendix 4 Protocol Deviations

Major protocol deviations will be summarized.

In general, the following list of major protocol deviations may have the potential to impact participants' rights, safety or well-being, or the integrity and/or result of the clinical study. Participants with major protocol deviations will be identified prior to database lock and the participants with major protocol deviations will be summarized by category.

- Developed withdrawal criteria but not withdrawn
- Entered but did not satisfy criteria
- Received a disallowed concomitant treatment
- Received wrong vaccination regimen or incorrect dose
- Other

Major protocol deviations which have a potential impact on immunogenicity will be flagged in the listings.

6.5. Appendix 5 Prior and Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms.

Based on their start and stop date, concomitant therapies will be reported in each applicable phase.

If a concomitant therapy record misses components of its start and/or stop dates (time, day and/or month and/or year):

- In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available, these will be compared to the month and the year of the periods, and the concomitant therapy record will be allocated to the period(s) where these date parts match. This rule may lead to assignment to multiple periods.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

There will be special attention to any systemic use of analgesics/antipyretics, started during [8] days following each vaccination (00:00 of day of vaccination + [7] days). Following ATC/DD codes will be used for this: N02A (OPIOIDS) and N02B (OTHER ANALGESICS AND ANTIPYRETICS), M01A (ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS) and M01B (ANTIINFLAMMATORY/ANTIRHEUMATIC AGENTS IN COMBINATION) (ATC/DD Index). The classes will be added in a footnote in all related tables and listings. For the use of analgesics/antipyretics which are taken on the day of vaccination, an exception is made in case the time is before vaccination. In this case, the concomitant medication is also allocated to the post-dose period.

6.6. Appendix 6 Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

Refer to the Appendix 5 of the CTP for Toxicity Grading Scale.

The laboratory values provided in Appendix 5 of the CTP serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

If a laboratory value falls within the grading as specified below but also within the local laboratory normal limits, the value is considered to be normal and will be reset to grade 0.

Ranges to convert FDA scale to SI units

Ranges to convert FDA scale to SI units		**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Albumin (g/L)	Hypo-albuminemia	28 - 31	25 - 27	< 25	
Eosinophils (10^9/L)		0.65 - 1.5	1.501 - 5.0	> 5.0	
Hemoglobin for male (g/L)		125 - 135	105 - 124	85 - 104	< 85
Hemoglobin for female (g/L)		110 - 120	95 - 109	80 - 94	< 80
Hemoglobin change from baseline (g/L)		Any decrease – 15	16 - 20	21 - 50	> 50
Lymphocytes (10^9/L)		0.75 - 1.0	0.5 - 0.749	0.25 - 0.499	< 0.25
Neutrophils (10^9/L)		1.5 – 2.0	1.0 – 1.499	0.5 - 0.999	< 0.5
Platelets (10^9/L)		125 – 140	100 – 124	25 – 99	< 25
Protein (g/L)	Hypo-proteinemia	55 – 60	50 – 54	< 50	
WBC (10^9/L)	Increase	10.8 – 15	15.001 – 20	20.001 – 25	> 25
	Decrease	2.5 – 3.5	1.5 – 2.499	1.0 – 1.499	< 1.0

Other Conversions

Blood, Serum, or Plasma Chemistries*		**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**	**Conversion Factor**
Glucose (mmol/L)	Hypoglycemia	3.61 – 3.83	3.05 – 3.60	2.50 – 3.04	< 2.50	/18.01477
	Hyperglycemia – Fasting	5.55 – 6.11	6.12 – 6.94	> 6.94		
	Hyperglycemia – Random	6.11 – 6.94	6.94 – 11.10	> 11.10		
Blood urea nitrogen (mmol/L)		8.2 – 9.3	9.4 – 11.1	> 11.1		
Creatinine (µmol/L)		133 – 150	151 – 177	178 – 221	> 221	/0.01131
Calcium (mmol/L)	Hypocalcemia	2.00 – 2.10	1.87 – 1.99	1.75 – 1.86	< 1.75	/4
	Hypercalcemia	2.62 – 2.74	2.75 – 2.87	2.88 – 3.00	> 3.00	/4
Magnesium (mmol/L)	Hypomagnesemia	0.53 – 0.62	0.45 – 0.52	0.37 – 0.44	< 0.37	/2.43072
Phosphorus (mmol/L)	Hypophosphatemia	0.74 – 0.81	0.65 – 0.73	0.52 – 0.66	< 0.52	/3.09693
Cholesterol (mmol/L)		5.20 – 5.43	5.44 – 5.82	> 5.82		
Coagulation						
Fibrinogen (µmol/L)	Increase	11.76 – 14.70	14.71 – 17.65	> 17.65		
	Decrease	4.41 – 5.88	3.68 – 4.40	2.94 – 3.67	< 2.94	/34

* Depending upon the laboratory used, reference ranges, eligibility ranges and grading may be split out by sex and/or age.

7. REFERENCES

Z. He, Y. Fong, Stat Med. 2019. Maximum Diversity Weighting for Biomarkers with Application in HIV-1 Vaccine Studies