Statistical Analysis Plan Amendment 3

Study ID: 222853

Official Title of Study: A phase 2a randomized, observer-blind, dose-finding study to evaluate the immunogenicity and safety of mRNA-based multivalent seasonal influenza vaccine candidates in adults 18 years of age and older

NCT number: NCT06431607

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TITLE PAGE

Protocol Title:	A phase 2a randomized, observer-blind, dose-finding study to evaluate the immunogenicity and safety of mRNA-based multivalent seasonal influenza vaccine candidates in adults 18 years of age and older
Study Number:	222853
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TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

VERSION HISTORY

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	24 May 2024	Clinical Study Protocol Amendment 1 (16 May 2024)	Not Applicable	Original version
Amendment 1	29 July 2024	Clinical Study Protocol Amendment 1 (16 May 2024)	Section 4.2.1: the consideration for analysis in YA & OA separately is moved under section 4.1 Section 4.5.1: iSRC added Section 4.6.1: added clarifications on the machine learning model Section 6.3.2.1: additional details on handling adverse events partial dates	To add minor updates to immunogenicity analyses and clarifications to the dose selection predictive modeling and handling adverse events partial dates
Amendment 2	10 January 2025	Clinical Study Protocol Amendment 2 (24 September 2024)	Section 1.2: Updated study design, design features, and study interventions Section 4.2.1: Added Table 4 for protocol amendment 2 Section 4.3.2.1: added clarification regarding	To align with protocol amendment 2, added minor clarifications in reactogenicity analyses and duration of events, and trademarks

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
			resolution for solicited AE Section 4.3.2.3: Updated section title Section 4.7: Added an additional interim analysis and clarifications for interim analyses Section 4.8: Updated in alignment with protocol amendment 2 Section 5: Updated sample size for part 2 and added clarifications for initial part in alignment with protocol amendment 2 Section 6.3.1.9: Updated to '>Grade 0' instead of '≥ Grade 0' Section 6.3.5: Added Flu D-TIV and Fluzone	
Amendment 3	10 Feb 2025	Clinical Study Protocol Amendment 2	Section 3: Added an additional analysis set for	To include additional analyses for safety laboratory assessments and

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
		(24 September 2024)	safety lab analyses Section 4.2.1: Updated CCI for FLU A/Michigan/173/ 2020 (H3N2) CCI in Table 4 Section 4.5.2: Added laboratory analysis Section 4.8: added changes to protocol defined analyses Section 6.3.2.4: Added handling of laboratory data	minor update to immunogenicity analyses

1. INTRODUCTION

The purpose of this SAP is to describe the planned analyses to be included in the CSR for Study 222853. Details of the planned analyses to support the Safety Review team (SRT), internal Safety Review Committee (iSRC), primary analysis, interim analysis as well as final analysis, are provided.

1.1. Objectives, Estimands and Endpoints

Table 1 Objectives and endpoints

Objectives	Endpoints1
Primary	
To evaluate the humoral immune response induced by the investigational study intervention	• CCI titer at Day 29 • Fold increase in CCI titer from Day 1 to Day 29 • CCI seroconversion from Day 1 to Day 29 • CCI at Day 1 and Day 29
Secondary	
To evaluate the humoral immune response induced by the investigational study intervention	In a subset[3] of participants: CCI
To evaluate the safety and reactogenicity profile of the investigational study intervention	• Occurrence of solicited administration site and systemic events within 7 days (i.e., from Day 1 to Day 7) after study intervention administration[4] • Occurrence of unsolicited AEs within 28 days (i.e., from Day 1 to Day 28) after study intervention administration[4] • Occurrence of SAEs[4] within 6 months (i.e., from Day 1 to Day 183) after study intervention administration • Occurrence of AESIs[4] within 6 months (i.e., from Day 1 to Day 183) after study intervention administration • Occurrence of MAAEs[4] within 6 months (i.e., from Day 1 to Day 183) after study intervention administration
Tertiary	
CCI	

Objectives	Endpoints1
CCI [redacted]	

Endpoints are designed for all the participants, unless indicated otherwise in the table.
AE: adverse event; AESI: adverse event of special interest; CCI [redacted]; MAAE: medically attended adverse event; MN: microneutralization; CCI [redacted] SAE: serious adverse event
[1] Refer to Table 2 for details of primary and secondary estimands.
[2] For all CCI [redacted] used to design the study interventions.
[3] Refer to Section 4.1 of the protocol for the description of subset.
[4] Refer to Section 8.3 of the protocol for the list of safety events and timeframe for collection

1.1.1. Estimands

Table 2 Study estimands

Ref #	Population	Objectives	Variable (Endpoint)	Intercurrent event (ICE)[1]		Population level summary
				Description	Handling strategy	
Primary						
1	Younger adults 18-64 YOA and older adults 65-85 YOA	To evaluate the humoral immune response induced by the investigational study intervention	• CCI [redacted] titer at Day 29 • Fold increase in CCI [redacted] titer from Day 1 to Day 29 • CCI [redacted] seroconversion from Day 1 to Day 29 • CCI [redacted] CCI [redacted] at Day 1 and Day 29	• Vaccination administration errors. • Taken prohibited medication or vaccination prior to Day 29 blood draw. • Day 29 blood draw taken out of allowed window.3 • Medical condition forbidden by protocol (i.e., either a confirmed immunodeficiency condition, new malignancy, or development of confirmed influenza disease) prior to Day 29 blood draw.	Hypothetical strategy: data from participant after ICE will be excluded from the analysis.	• Between-group geometric mean titer (GMT) ratio (Flu mRNA candidate over Comparator) with 95% confidence interval (CI) • Within-group geometric mean increase (GMI) with 95% CI • Between-group difference in seroconversion rate (SCR[4]) (Flu mRNA candidate minus Comparator) with 95% CI • Between-group difference in CCI [redacted] CCI [redacted] (Flu mRNA candidate minus Comparator) with 95% CI

Ref #	Population	Objectives	Variable (Endpoint)	Intercurrent event (ICE)[1]		Population level summary
				Description	Handling strategy	
	Secondary					
2	Refer to 1	To evaluate the humoral immune response induced by the investigational study intervention	CCI [redacted]	Refer to 1	Refer to 1	Refer to 1
3	Refer to 1	To evaluate the safety and reactogenicity profile of the investigational study intervention	• Occurrence of solicited administration site and systemic event within 7 days (i.e., from Day 1 to Day 7) after study intervention administration • Occurrence of unsolicited AEs within 28 days (i.e., from Day 1 to Day 28) after study intervention administration • Occurrence of SAEs within 6 months (i.e., from Day 1 to Day 183) after study intervention administration • Occurrence of AESIs within 6 months (i.e., from Day 1 to Day 183) after study intervention administration • Occurrence of MAAEs within 6 months (i.e., from Day 1 to Day 183) after study	Not applicable	Treatment policy: all data collected will be included in summaries.	Proportion of participants who report each of the endpoint. No treatment comparison is planned.

Ref #	Population	Objectives	Variable (Endpoint)	Intercurrent event (ICE)[1]		Population level summary
				Description	Handling strategy	
			intervention administration			

Endpoints are designed for all the participants, unless indicated otherwise in the table.

AE: adverse event; AESI: adverse event of special interest; CI: confidence interval; GMI: mean geometric increase; GMT: geometric mean titer; CCI ; ICE: intercurrent event; MAAE: medically attended adverse event; CCI SAE: serious adverse event; SCR: seroconversion rate; CCI CCI YOA: years of age.

[1] Refer to Section 9.2 of the protocol for more information on intercurrent events.

[2] All CCI used to design the study intervention.

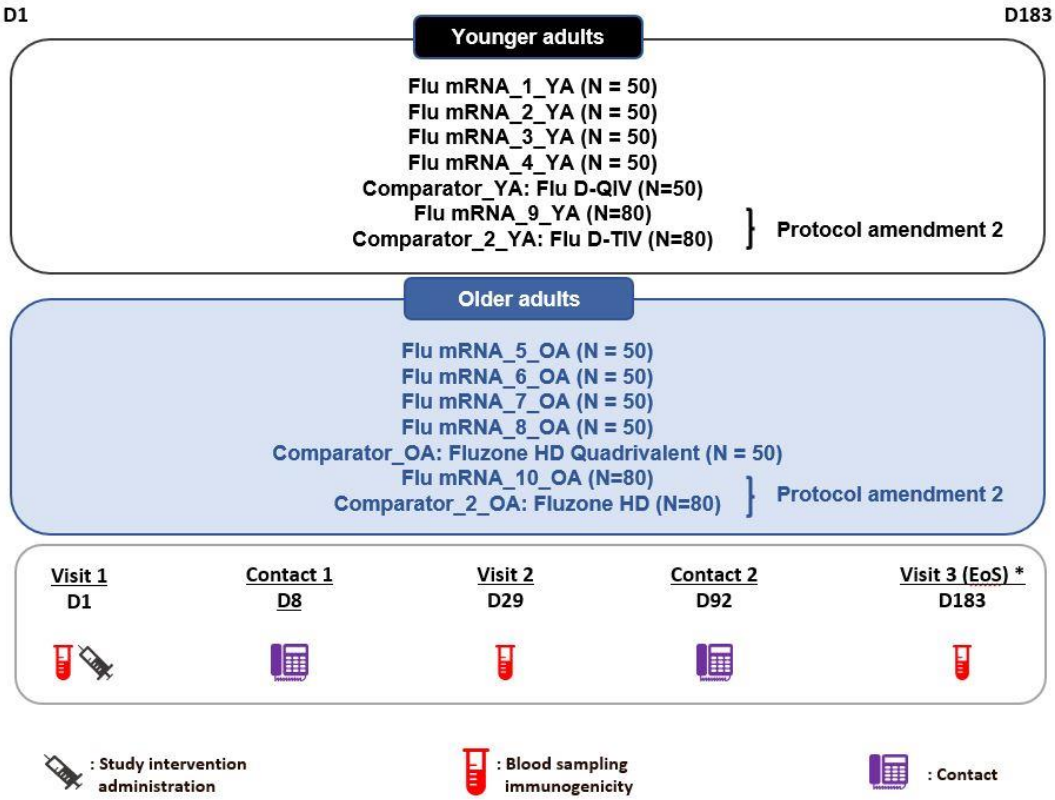
[3] Study discontinuation, blood sample taken out of window and missing result at pre- or post-dose are not considered intercurrent events and will be handled as missing data. More details will be provided in the statistical analysis plan (SAP).

[4] Refer to Section 9.3.1 of the protocol for definition of SCR and CCI

[5] Refer to Section 4.1 of the protocol for the description of subset.

1.2. Study Design

Overview of Study Design and Key Features



D: day; N: number of participants.

*Visit 3 will be replaced by a Contact if participant has received the standard of care (SoC) vaccination against seasonal flu after Visit 2 and prior to this visit and did not report any adverse events that would require physical examination on site.

Design Features	– Study phase: 2a – Single-country, multi-center – Self-contained – Intended duration of the study per participant: approximately 6 months – Study population: Healthy or medically stable participants aged 18-64 and 65-85 years of age (Refer to Section 5 of the protocol for full description of study population) – For the initial part of the study: 5 groups enrolled in parallel for younger adults (YAs) (4 Flu mRNA groups and 1 comparator group) and 5 groups enrolled in parallel for older adults (OAs) (4 Flu mRNA groups and 1 comparator group). For protocol amendment 2: 2 groups enrolled in parallel for YAs (1 Flu mRNA group and 1 comparator group) and 2 groups enrolled in parallel for OAs (1 Flu mRNA group and 1 comparator group). – Planned (approximate) number of participants to be enrolled: 250 YAs (50 participants per group) and 250 OAs (50 participants per group) in the initial part of the study, and 160 YAs (80 participants per group) and 160 OAs (80 participants per group) in protocol amendment 2. – Blinding: observer-blind. Refer to Section 6.4 of the protocol for more information on blinding. – A subset representing approximately 50% of the total study population, randomly selected and equally distributed over the groups, will be defined for this study. This subset will be used to further characterize the humoral immune response, i.e., virus microneutralization (MN) titers CCI [redacted] Refer to Section 6.3 of the protocol for more details. – Aspects of data collection: blood samples, safety events. – Method of data collection: o Standardized electronic Case Report Form (eCRF) o Solicited events and the occurrence of unsolicited AEs will be collected using an electronic Diary (eDiary). Participants, with support from the site, will install the eDiary application on their own personal, handheld device (e.g., mobile phone, tablet) or the site will provide a device that is pre-programmed with the eDiary. o The collection of blood samples will be documented with a validated digital collection application for sample management. – Safety monitoring: A blinded medical monitor, designated Safety Lead (or delegate) and Safety Review Team (SRT) will continuously monitor available safety data throughout the study. If a potential safety concern/signal is identified that requires unblinded review, an unblinded internal Safety Review Committee (iSRC), independent from the project, will review available safety data. Refer to Section 8.3.6 of the protocol for more information on safety monitoring strategy applicable to this study and to Section 10.1.6 of the protocol for SRT and iSRC composition and role. – Investigational study interventions CCI [redacted] CCI [redacted] CCI [redacted] Refer to Section 6.1 of the protocol for more details on the composition of study interventions to be administered. – A primary completion analysis (PCA) is foreseen and will be conducted upon availability of all primary and secondary endpoints up to Day 29 for participants CCI [redacted]. A restricted study team will be unblinded to individual participant data and all efforts will be made to ensure the participants, investigators and monitoring staff blinding is maintained up to study end. The rest of the study team will have access to aggregated unblinded summaries but will remain blinded to individual participant data and treatment assignment until the end of the study. It is possible, however, due to the limited sample size, that unblinding occurs for a few participants having a specific AE or SAE (e.g., an

	AE/SAE occurring only in a single participant). Therefore, anyone having access to the results could become unblinded regarding those few specific cases.
Study intervention	All study interventions are administered as single-dose intramuscularly (IM). – GSK's Flu Dresden-Quadrivalent Influenza Vaccine, hereafter referred to as Flu D-QIV (commercially available as Fluarix Quadrivalent in the US). – Sanofi's Fluzone High-Dose Quadrivalent Vaccine, hereafter referred to as Fluzone HD (commercially available as Fluzone High-Dose Quadrivalent in the US). – GSK's Flu Dresden – Influenza Vaccine, hereafter referred to as Flu D-TIV (commercially available as Fluarix in the US). – Sanofi's Fluzone High-Dose Vaccine, hereafter referred to as Fluzone HD (commercially available as Fluzone High-Dose in the US).
Study intervention Assignment	All participants will be centrally randomized to study intervention using a Randomization and Trial Supply Management (RTSM). Study intervention will be assigned randomization number (study intervention number) and dispensed at Visit 1. The randomization order of study intervention assignment is created by a GSK Randomization Generation system. A stratified and permuted block approach is employed to maintain equal study intervention balance within each stratum. In addition, a subset representing approximately 50% of the total study population, will be randomly selected and equally represented by study intervention within stratum CCI [redacted]
Interim Analysis	Additional analyses e.g., safety analyses, may be conducted to support the SRT and iSRC decisions, as described in Section 8.3.6 of the protocol. Refer to Section 10.1.6 of the protocol for the SRT and iSRC roles.

2. STATISTICAL HYPOTHESES

There is no formal hypothesis testing in this study; where statistical methods are applied, the emphasis will be on estimation with 95% confidence intervals (CIs).

2.1. Multiplicity Adjustment

As this study is descriptive, no adjustment for type 1 error will be done.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened	All participants who were screened for eligibility	Study Population
Enrolled	Participants who received the study intervention, had an immunogenicity blood draw at pre-dose or were randomized. Note that as per good clinical practice (GCP) enrolled participants should have completed the informed consent process and participants should be eligible before initiating any study procedure. Note: screening failures (who never passed screening, even if rescreened) and participants screened but never enrolled into the study (met eligibility but not needed) are excluded from the Enrolled analysis set as they did not enter the study. Analysis will be based on the intervention that participants are randomized to.	Study Population
Exposed	All participants who received the study intervention. Analysis per group will be based on the actual study intervention that participants received.	Safety

Analysis Set	Definition / Criteria	Analyses Evaluated
Per Protocol Set (PPS)	All eligible participants who received a study intervention dose as per protocol, had immunogenicity results at both Day 1 and Day 29 for at least 1 vaccine CCI , complied with Day 1 and Day 29 blood draw intervals (refer to Table 9), without intercurrent conditions* that may interfere with immunogenicity and without prohibited concomitant medication/vaccination before Visit 2 (Day 29). Immunogenicity data at Day 183 will be censored in case of non-compliance with the blood sample visit, in case intercurrent conditions* that may interfere with immunogenicity have occurred, or if prohibited concomitant medication/vaccination were taken before Day 183. The analysis will be done according to the actual study intervention that participants received.	Demography, Immunogenicity
Safety Laboratory set	All participants in the Exposed analysis set who had a least one safety laboratory test result at Day 1 and Day 29.	Safety

* Note: Intercurrent medical conditions that may lead to elimination of immunogenicity results from the PPS are the occurrence of either a confirmed immunodeficiency condition, new malignancy, or development of confirmed influenza disease.

4. STATISTICAL ANALYSES

4.1. General Considerations

All statistical analyses will be done for OA and YA separately, except when indicated otherwise.

Missing data will not be imputed unless mentioned otherwise (refer to Section 6.3.2).

Participants will be analyzed according to the age group, country, and flu vaccination history in preceding 2 years strata that was used at the time of randomization but with the actual vaccine received.

4.1.1. General Methodology

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

Continuous data will be summarized using descriptive statistics (n, mean, standard deviation, median, interquartile range, minimum, and maximum). Categorical data will be summarized using the participant count and percentage for each category.

A row denoted “Missing” will be included in count tabulations where specified on the shells to account for dropouts and missing values.

The denominator for all percentages will be the number of participants with non-missing values of corresponding parameter in that study group within the analysis population of interest, unless otherwise specified.

Confidence intervals (CIs) will use 95% confidence levels.

For calculations regarding functional titers, values reported as below a quantifiable threshold will be replaced by half the threshold while values above a quantifiable threshold will be assigned the value of the threshold.

A table, figure, listing (TLF) is to be generated for any required item even where no data is available or reported. In such cases, except for table presenting percentage of participants which will show 0%, the table, figure, or listing will state: "No Data Reported". This will confirm to the health authorities that all data for the tables, figures, listings, and narratives are accounted for.

4.1.2. Baseline Definition

Baseline will be defined as the last non-missing evaluation prior to study intervention administration, unless otherwise specified.

4.1.3. Definitions

Abbreviation/term	Definition
CCI SCR	The percentage of dosed participants who have either a CCI pre-dose titer <1:10 and a post-dosing CCI titer CCI or a pre-dose CCI titer CCI and at least a CCI increase in post-dosing CCI titer.
CCI SCR	CCI
CCI	The percentage of dosed participants with a CCI ≥1:40.

CCI SCR: seroconversion rate; CCI

4.2. Primary Endpoint(s) Analyses

4.2.1. Immunogenicity Endpoints

The primary analyses of immunogenicity endpoints will be based on the PPS. The group differences between each investigational study intervention candidate and the comparator group will be assessed separately as follows for each CCI :

- At each post-dosing timepoint and for each age group separately, the 2-sided CI for between-group GMT ratio between investigational study intervention and (over) the comparator group will be derived from an ANCOVA model on $\log_{10}$ transformed concentration. The ANCOVA model will include group (i.e., each of the investigational study intervention and the comparator group), actual age of participants, country, flu vaccination history in the preceding 2 years and $\log_{10}$-transformed titer at pre-dosing as fixed effects. The adjusted GMT and GMI in each group will be obtained from the same model with 95% CI. Missing data will not be replaced.
- For a given age group, the 2-sided 95% CI for the group difference in SCR between an investigational study intervention and (minus) the comparator group will be computed at Day 29 based on the method of Miettinen and Nurminen [Miettinen, 1985]

The percentage of participants achieving CCI [redacted], defined as having post-dosing titer CCI [redacted] will be summarized by age group with associated exact 95% CI.

All analyses will be performed separately for each age group, unless otherwise indicated.

The following SAS code will be used for computation of GMT ratio where AVAL and BASE are the log10 transformed GMT values at pre-dose and post-dose respectively:

```
PROC GLM DATA=immuno ALPHA=0.05;
    CLASS trt01a;
    BY agecat strain;
    MODEL aval = trt01a country vacchist base age;
    LSMEANS trt01a / CL;
RUN;
```

For the purpose of analysis, previous flu vaccination history reported as 'Unknown' will be classified as 'No'.

The SCR will be computed using the following SAS code where AVALC is the seroconversion status post-dose:

```
PROC FREQ DATA=immuno;
      BY agecat strain;
      TABLE trt01a*avalc / RISKDIFF (CL=MN) ALPHA=0.05;
RUN;
```

The assays used in this study CCI [redacted] are presented in Table 3 for protocol amendment 1 and Table 4 for protocol amendment 2.

CCI

[redacted]

CCI

CCI

4.3. Secondary Endpoint(s) Analyses

4.3.1. Immunogenicity Secondary Endpoint(s)

To evaluate the humoral immune response induced by the investigational study intervention. For a subset of participants (50% of the total study population), the CCI titer for each strain on Day 29 will be assessed as follows:

CCI

[redacted]

4.3.1.1. Main analytical approach

The analyses of secondary endpoints will be performed as described in Section 4.2.1 except for seroprotection.

4.3.2. Reactogenicity and Safety Endpoints

The analysis of reactogenicity and safety endpoints will be based on the Exposed Set. All analyses will be done separately for OAs and YAs, unless specified otherwise.

4.3.2.1. Solicited AEs

All solicited events must be recorded into the eDiary during the first 7 days following administration of the dose of study intervention (Day 1 to Day 7 included), irrespective of occurrence or intensity. Solicited events ongoing after Day 7 will continue to be recorded into the eDiary and followed-up until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, the participant is lost to follow up, or study is completed.

The following administration site events will be solicited:

- Pain
- Redness
- Swelling
- Lymphadenopathy, defined as localized axillary, cervical or supraclavicular swelling or tenderness ipsilateral to the injection arm.

The following systemic events will be solicited:

- Fever
- Chills
- Headache
- Fatigue (tiredness)

- Myalgia (muscle joint)
- Arthralgia (joint pain)

Note: Participants will be instructed to measure and record the temperature in the evening. If additional temperature measurements are taken at other times of the day, participants will be instructed to record the highest temperature in the eDiary. The preferred location for measuring temperature will be the oral cavity.

Grading or actual temperature/redness and swelling will be captured in the eDiary as per a modified grading of symptoms based on the FDA toxicity grading guidance for industry [FDA, 2007] with Grades 3 and 4 combined except for fever (refer to Section 6.3.2.2). When available, information related to solicited events (e.g., occurrence, grading) collected by study staff when daily eDiary is missing/incorrect will be used in these summaries.

The percentage of participants reporting each individual solicited administration site event (any grade, Grade ≥2, Grade 3 and MAAEs) and solicited systemic event (any grade, Grade ≥2, Grade ≥3 and MAAEs) within the 7-day follow up period (i.e., Day 1-Day 7 post-dosing) will be tabulated for each group according to the maximum grade in Day 1 until resolution (i.e. first occurrence of grade=0).

The same summaries will be generated overall by category i.e., for any administration site solicited events, any systemic solicited events, and any solicited events.

The duration of solicited AEs of any grade (see Section 6.3.1.9) will be summarized. The start date is the first day during the 7-day solicitation period with the symptom at grade > 0 while the stop date is the last day with the symptom at grade > 0 in or beyond the solicited period. In addition, the duration for specific grade(s) for each symptom defined as the number of days in the reporting period with grade above or equal to specific grade will be summarized.

Prolonged solicited AEs that continue beyond Day 7 or Day 28 will be identified using a flag in listing of AEs.

The number of completed eDiary days in the solicited period for a participant will be summarized by study group using frequency table.

4.3.2.2. Unsolicited AEs

An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

The verbatim reports of unsolicited AEs will be reviewed by a qualified person and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) Dictionary for Adverse Reaction Terminology. Every verbatim term will be matched with the appropriate PT.

A study intervention causally related AE is defined as an AE for which the investigator classifies the possible relationship to study intervention as "Yes". Investigators will not be required to assess the causality of solicited AEs if the onset is during the solicitation period.

The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE/device deficiency. The investigator will use clinical judgment to determine the relationship.

The percentage of participants with any unsolicited AEs within the 28-day follow up period (i.e., Day 1 to Day 28 post-dosing) with its exact 95% CI will be tabulated by group and by MedDRA PT and System Organ Class (SOC). Similar tabulation will be done for Grade 3 unsolicited AEs, for fatal AEs, for any causally related unsolicited AEs, for Grade 3 causally-related unsolicited AEs, for any causally related fatal AEs, and for unsolicited AEs resulting in a medically-attended visit.

In the AE summaries, a participant with 2 or more AEs within the same SOC or PT level but different relationship will be counted only once in the level using the related incident.

4.3.2.3. Adverse Events of Special Interest, Serious Adverse Events, and Medically Attended Adverse Events

The following events are considered as AESIs in this study.

- Potential immune-mediated disease (pIMDs)
- Severe hypersensitivity reactions within 24 hours after study intervention administration
- Myocarditis/Pericarditis. Refer to Section 8.3.4 of the protocol for myocarditis/pericarditis assessment and definitions.

The summary of event characteristics will be provided for each AESI as well as any potential immune-mediated diseases [pIMDs] (new onsets and exacerbations) including number and percentage of participants with any event. In addition, summary will be provided by relationship to study intervention, maximum grade/severity, outcome, and the action taken. The worst-case approach will be applied at participant level for the maximum grade/severity, i.e., a participant will only be counted once as the worst case from all the events experienced by the participant. For action taken for an event, a participant will be counted once under each action.

The percentage of participants with at least 1 report of SAE (any, related, fatal and fatal related), with at least 1 report of MAAE and with at least 1 report of AESI, respectively, classified by the MedDRA SOC and PT and reported from Day 1 up to study end will be tabulated with exact 95% CI.

In the AESI/SAE summaries, a participant with 2 or more AEs within the same SOC or PT level but different relationship will be counted only once in the level using the related incident.

CCI

4.5. Safety Analyses

Refer to Section 4.3.2.

4.5.1. Safety Review Team (SRT) & Internal Safety Review Committee (iSRC)

SRT and iSRC will be appointed for the study for safety monitoring.

The SRT will receive blinded summaries of safety data that will be generated by the independent Data Analysis Centre (iDAC) who will have access to the randomization scheme. The iSRC will receive unblinded summaries of safety data that will be generated by the iDAC. The blinded summaries will include all results from the safety analysis by

age group while the unblinded summaries will include all results from the safety analysis by age and study group.

The pausing criteria are defined in Table 5. Once a pausing criterion is confirmed to be met, the Sponsor will inform the investigator(s), triggering a pause in further dosing in all sites and at all dose levels in all study groups until iSRC has reviewed the relevant data. Pausing criteria will be assessed by the iSRC and/or SRT as applicable during the safety evaluations of the data. Meeting any of these pausing criteria will trigger a hold of study intervention administration irrespective of number of participants enrolled and/or timing of the event, for all the study groups.

If a pausing criterion was met or safety signal observed during the SRT or iSRC meeting, all dose administrations will cease immediately, but all other procedures relating to safety and immunogenicity assessments will continue. Following additional assessment by the SRT or iSRC, a decision to continue, suspend or modify the conduct of the study will be taken by the committee.

The following statistical analysis will be conducted on pausing criteria:

- Tabular listings for all pausing criteria together will be generated and include information on study group, participant number, pausing criterion, dose, and details including the severity grade, event outcome etc.
- Detailed listings on any event related to any pausing criterion will be generated for each pausing criterion.
- Number and percentage of participants meeting each pausing criterion will be tabulated with exact 95% CI. This analysis will be done by group and after each dose.

Table 5 Study Pausing Criteria

Pausing criteria	Events	Number of participants per dose & individual investigational study group (i.e., non-licensed vaccines)
1a	Death or any life-threatening SAE for which there is no alternative attributable cause as per Investigator assessment	≥1
1b	Any non-life-threatening SAE that is considered related to vaccination as per Investigator or Sponsor assessment	≥1
1c	Necrosis at the injection site	≥1
1d	Confirmed myocarditis or pericarditis events not clearly attributable to any cause other than study vaccine	≥1
1e	Anaphylaxis, unless clearly attributable to a cause other than study vaccine	≥1

2a	Similar (Preferred Terms in same HLT based on MedDRA coding) Grade 3 unsolicited AEs, considered related to vaccination as per Investigator assessment, with an event onset within the specified period of follow-up for unsolicited events	≥2

[1] After the first iSRC meeting triggered by the occurrence of this pausing criterion, each additional participant meeting this pausing criterion will thereafter trigger a pause and iSRC review.

[2] After the first iSRC meeting triggered by the occurrence of a given pausing criterion for a given event, the iSRC will indicate the conditions under which it requires further notification and review of the subsequent same/similar events.

The TFLs to be generated to support SRT and iSRC reviews will be detailed in a dedicated output programming specification (OPS).

4.5.2. Laboratory Data

The analysis of biochemistry and auto immune hepatitis (AIH) laboratory tests collected on Day 29 samples will be based on the Safety Laboratory Set. As biochemistry laboratory testing on frozen samples has limitations because samples frozen for prolonged periods of time over 3 months may show lower activity/ values, not all samples may be eligible for testing. All analyses will be done separately for OAs and YAs, unless specified otherwise.

The following laboratory assessments will be performed:

Assay type	System	Component
Biochemistry	Serum	Bilirubin, Direct
		Bilirubin, Total
		Bilirubin, Indirect
		Creatine Phosphokinase (CPK)
		Gamma Glutamyl Transferase (GGT)
		Alanine aminotransferase (ALT)
		Aspartate aminotransferase (AST)
		Alkaline Phosphatase (ALP)
***Auto Immune Hepatitis (AIH)**		Antinuclear Antibody
		Anti-Smooth Muscle Antibody
		Anti-Mitochondrial Antibody
		Liver-Kidney Microsomal Antibody

*Conditional testing only for samples with abnormal ALT, AST, and bilirubin with grade greater than or equal to 1.

Summaries of the grade increase from baseline grade will be provided for all biochemistry and AIH parameters that are gradable by FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. These summaries will display the number and percentage of participants with a grade increasing on Day 29 from their baseline grade. Any increase in grade on Day 29 from baseline will be summarized along with any increase to grade 3 and any increase to grade 4. When no FDA toxicity grading is defined for a specific parameter, increase from normal values at baseline (Day 1) to abnormal values on Day 29 will be provided. Non-

missing laboratory results that are within normal range will be denoted as grade 0. Unless otherwise stated, no derivation of missing data will be performed and the grade of missing laboratory results will be set to missing.

Box plots of change from baseline of biochemistry and AIH laboratory tests at Day 1 and Day 29 by study treatment will be provided using median and quartiles (Q1, Q3, Q3-1.5 IRQ, Q3+1.5 IRQ).

A supportive listing of the biochemistry and AIH data, including the age group, study treatment group, participants ID, laboratory test name and result, and date and time of measurements, will be generated.

4.6. Other Analyses

4.6.1. Dose selection

To leverage the data accumulated in this study and in study 217884, a predictive modelling will be used to inform the final formulation that will be tested in Phase 3 studies in younger and older adults. The goal is to identify an optimal dose of individual components of the mRNA vaccine that would lead to an appropriate immunological response in terms of group GMT ratio in comparison to the control vaccines across strains.

A pooling of the data from studies FLU SV MRNA-002 (217884) and FLU SV MRNA-024 (222853) including only the investigation (mRNA) groups from both studies will be used for the modelling. Imputation of missing values will not be performed i.e., only participants with non-missing pre-dose and post-dose immunogenicity data will be included. Based on study 217884, < 3% of the records are expected to be excluded due to missing values.

For this analysis, a meta-learner consisting of the following statistical and machine learning models will be used:

- Penalized linear regression model: This model assumes an additive linear dose-effect relationship between the dependent variable (log10 transformed immune response) and the features (including pre-vaccination titer, study/phase, strain, age, age group, sex, height, weight, country, flu vaccination history in the preceding 2 years, amount of each antigen in the mRNA vaccine received). The input hyperparameters include the penalty parameter (which determines strength of the regularization parameter) and mixture parameter (which determines the type of regularization [ridge, lasso, elastic net] to be applied). As a result of these regularizations, some feature selection may be induced (i.e., features entirely excluded from the model).
- Spline regression model: To account for the possible non-linearity in the dose-response relationship between the dependent variable and continuous features, the functional form of continuous features in the linear predictor will be modelled using B-splines. The same features used for linear regression model will be considered. In addition to the input hyperparameters for penalized linear regression, additional parameters will include number of knots (determines the degrees of freedom for the

b-spline; as this increases, more flexible and complex curves can be generated) and degree (determines the degree of the piece-wise polynomial).

- Linear mixed model: Considering that the independence assumption of the linear model may not be met due to the hierarchical nature of the data (grouped by participant) and the within-subject correlation expected in such data, we propose including a mixed-effects model to account for this correlation structure. For this model, a random intercept is foreseen in addition to the fixed effects for pre-vaccination titer, study/phase, strain, age, age group, sex, height, weight, country, flu vaccination history in the preceding 2 years, amount of each antigen in the mRNA vaccine received. For this model, no input hyperparameters are applicable.
- Random forest model: This is a non-parametric model based on regression trees. This model makes no assumption on the linearity of dose-effect relationship between the dependent variable and features hence making it suitable for a potentially non-linear relationship. In addition, it allows for higher-order interactions. The dependent variables and features are as listed for linear regression model. The input hyperparameters include the number of predictors that will be randomly sampled at each split when creating the tree models (mtry), the number of trees contained in the ensemble (trees) and the minimum number of data points in a node that are required for the node to be split further (min_n).

For our final model, we propose an ensemble model (a meta-learner) which leverages the strengths of the individual models potentially leading to a better prediction performance.

The following steps are used to obtain predictions from the ensemble model:

- Obtaining model-specific predictions from the training set: this step involves assembling the assessment set predictions for the training set from each candidate model. The features will include the prediction of each model candidate with a unique feature corresponding to each combination of hyperparameters.

Figure 1 shows an example with the dependent variable (log_aval=log10 transformed immune response) and the features (predicted values for a subset of candidate models).

- Create a meta-learner model to blend these predictions: this involves the combination of the candidate models using a penalized linear model. Considering the possibility of a high correlation between predictions from the candidate models, a lasso penalty with input parameters including penalty and mixture as applicable will be used. This step determines the weight of the candidates in the final ensemble model and may result in a single candidate model being identified as the best prediction model.
- For each member of the ensemble, fit the model on the full training set: this involves fitting each of the candidate models included in the ensemble accounting for the final weights i.e., only the candidates with non-zero weight are retained and fitted in this step.

Figure 1 Stacking of candidate models prior to ensembling.

	log_aval	basic_linear_reglter4	basic_linear_reglter6	basic_linear_reg_1_5	basic_linear_reglter8
1	3.107	3.0708	3.0678	3.0695	3.0684
2	2.204	2.2179	2.2042	2.2174	2.2071
3	3.573	3.5458	3.5485	3.5481	3.5470
4	2.796	2.7950	2.7997	2.7961	2.7964
5	2.806	2.8061	2.8076	2.8074	2.8065
6	3.303	3.2864	3.3074	3.2824	3.3020
7	2.204	2.2218	2.2205	2.2218	2.2210
8	3.278	3.2641	3.2592	3.2665	3.2599
9	2.505	2.4946	2.4900	2.4923	2.4905
10	2.204	2.2057	2.2049	2.2039	2.2051
11	3.546	3.5251	3.5210	3.5266	3.5206
12	3.055	3.0471	3.0474	3.0484	3.0453
13	2.204	2.2189	2.2308	2.2197	2.2273
14	3.144	3.1383	3.1549	3.1344	3.1504
15	1.903	1.9300	1.9319	1.9314	1.9320

A split of 80% training set and 20% test set of all data including both age groups will be performed. This split will involve a random selection with subject as grouping factor and with age group, study and phase as stratification factors i.e., a subject will only appear in either the training or test set and the two age groups, study and phase will be equally represented in the training and test sets. A summary of the independent variables used for the model will be provided for the training and test sets to assess balance between sets,

A k-fold cross-validation framework will be applied to the 80% of the pooled data (i.e., the training set) for the purposes of hyperparameter tuning and assessment of candidate models. Specifically, we will use a repeated 10-fold cross-validation approach, where the training set is randomly divided into 10 folds. In each iteration, 9 folds are used for training and the remaining fold is used for assessment. This process will be repeated 3 times, providing a total of 30 performance metrics for each candidate model [Kuhn M, 2013], which will be averaged to derive the overall performance metric for the candidate model.

For parameter tuning, the Bayesian optimization technique based on gaussian process will be used [Koehrsen, 2018; Frazier, 2018; Shahriari, 2016]. Using expected improvement as the acquisition function, this approach attempts to select the best set of hyperparameters that minimize the performance metric (i.e., root mean squared error [RMSE]).

The 20% test set will be used for the evaluation of the final ensemble model. The predictive ability of this model will be assessed based on the RMSE on the test set which will be expected to be similar to the performance of the best candidate model on the training set.

The global feature importance of the models will also be assessed to determine the level of importance of the contributing features. This will be done using the feature permutation approach. This approach involves permuting or shuffling the values of a feature, predicting from the model, and then measuring how much worse the model fits the data compared to before shuffling [Breiman, 2001] in terms of the performance metric (RMSE).

The final model will be used to predict the post-dose GMT for participants from each age-appropriate control group assuming they would have received the new candidate mRNA formulations.

The t-test 95% confidence interval for the paired observed and predicted values will be used to obtain the CI for group GMT ratio.

The candidate mRNA formulations that exhibit higher GMT ratios for most of the antigens, will be considered more effective as it pertains to identification of the optimal formulation. In the case that multiple formulations exhibit similar estimated GMT ratios, the final decision on the optimal formulation will be made by clinical/safety team considering all available data.

The total dose of the recommended formulation will not be higher than the highest dose used in the two above-mentioned clinical studies and will be within the range of doses considered to have acceptable reactogenicity and safety profile by the safety review team, considering the total mRNA amount and other available data.

The final model and result of the prediction for all potential doses will be presented in the study report. All modelling will be done using the *tidymodels* and where necessary, other available packages in R.

4.6.2. Subgroup analyses

No subgroup analyses are planned.

4.7. Interim Analyses

In addition to the analyses detailed below, additional analyses, e.g., safety analyses, may be conducted to support the SRT and iSRC decisions, as described in Section 8.3.6 of the protocol. Refer to the Section 10.1.6 of the protocol for the SRT and iSRC roles.

As this study is descriptive and each analysis will be completed on expected final immunogenicity data, there will be no type I error adjustment for the different analyses. All interim analyses will be based on the Exposed Set.

4.7.1. Sequence of interim analyses

A primary completion analysis (PCA) is foreseen and will be conducted upon availability of all primary and secondary endpoints up to Day 29 for participants enrolled in parallel in the 4 Flu mRNA groups and 1 comparator group per age category of the initial part of the study CCI

An interim analysis will be conducted upon availability of all primary and secondary endpoints up to Day 29 for participants enrolled in parallel in 1 Flu mRNA group and 1 comparator group per age category of protocol amendment 2 to enable consolidation of the immunogenicity and reactogenicity data package for the selected dose formulation CCI An additional interim analysis will be performed based on availability of safety endpoints up to last visit (Day 183) for participants enrolled in the initial part of the study.

A restricted study team will be unblinded to individual participant data and all efforts will be made to ensure the participants, investigators and monitoring staff blinding is maintained up to study end. The rest of the study team will have access to aggregated unblinded summaries but will remain blinded to individual participant data and treatment assignment until the end of the study. It is possible, however, due to the limited sample size, that unblinding occurs for a few participants having a specific AE or SAE (e.g., an AE/SAE occurring only in a single participant). Therefore, anyone having access to the results could become unblinded regarding those few specific cases.

Note that the Day 29 interim analysis will be triggered once the planned number of subjects have had up to Day 29 safety follow-up and immunogenicity blood draw collected. Considering potential over-enrolments, all other participants not included in this analysis, will be included in the final analysis.

A final analysis with all primary and secondary endpoints obtained until the last visit (Day 183) will be performed and made available to the investigators and submitted to regulatory authorities, as appropriate.

Analysis of tertiary endpoints may be performed at a later stage.

4.8. Changes to Protocol Defined Analyses

Changes from the originally planned statistical analysis specified in the protocol are detailed in Table 6.

Table 6 Changes to Protocol Defined Analysis Plan

Protocol Defined Analysis	SAP Defined Analysis	Rationale for Changes
• Laboratory evaluations including the analyses of hematology laboratory tests and biochemistry laboratory tests are not defined as objectives and endpoints in protocol amendment 2.	• All safety lab analyses will be performed on the Safety Lab analysis set. All available data, including changes in laboratory grades, and other safety parameters will be evaluated by study treatment group.	• To support US FDA query regarding safety labs from study mRNA Combo-001.

5. SAMPLE SIZE DETERMINATION

The primary objective is to explore how the different candidates of Flu mRNA vaccine compare to licensed comparator group(s).

In the initial part of the study, the sample size of 47 evaluable participants in each dose level cohort and age group, is expected to provide a 95% CI for group difference in SCR with a lower half-width ≤ 20% for difference in SCR for the different strains (Table 7) and a 95% CI with an expected half-width of 1.6 fold for the group GMT ratio considering a population standard deviation of 0.5 in $\log_{10}$ transformed GMT post-dosing and 5% of unevaluable participants per group.

Table 7 Half-width of 95% CI for the group difference in seroconversion rates by comparator and CCI (Evaluable N per group = 47)

Comparator	CCI	Proportion (%)*	95% CI of Proportion (%)	Lower Half-width of 95 CI (%)
Flu D-QIV	[redacted]	59.3	57.5; 61.1	20
Flu D-QIV	[redacted]	76.2	72.3; 79.6	18
Flu D-QIV	[redacted]	68.3	63.6; 72.8	19
Fluzone HD Quadrivalent	[redacted]	43.3	39.4; 47.3	19
Fluzone HD Quadrivalent	[redacted]	51.0	48.3; 52.9	20
Fluzone HD Quadrivalent	[redacted]	62.0	50.1; 71.8	20

CCI [redacted]

CCI [redacted]

CCI [redacted]

Flu D-QIV: GSK's Flu Dresden-Quadrivalent Influenza Vaccine; Fluzone HD: *Sanofi's Fluzone High-Dose Quadrivalent Vaccine.*

In part 2 of the study (protocol amendment 2), a sample size of 76 evaluable participants in each study group and age group, is expected to provide a 95% CI for group difference in SCR with a lower half-width ≤ 16% for difference in SCR for the different strains (Table 8) and a 95% CI with an expected half-width of 1.45 fold for the group GMT ratio

considering a population standard deviation of 0.5 in $\log_{10}$ transformed GMT post-dosing and 5% of unevaluable participants per group. The increased sample size is aimed at increasing the level of precision for the estimated CIs.

Table 8 Half-width of 95% CI for the group difference in seroconversion rates by comparator and CCI in part 2 of the study (protocol amendment 2) (Evaluable N per group = 76)

Comparator	CCI	Proportion (%)[1]	95% CI of Proportion (%)[1]	Lower Half-width of 95 CI (%)
Flu D-TIV		59.3	57.5; 61.1	16
Flu D-TIV		76.2	72.3; 79.6	14
Flu D-TIV		68.3	63.6; 72.8	15
Fluzone HD		43.3	39.4; 47.3	15
Fluzone HD		51.0	48.3; 52.9	16
Fluzone HD		62.0	50.1; 71.8	16

CCI

CCI

CCI

Flu D-QIV: GSK's Flu Dresden-Quadrivalent Influenza Vaccine; Fluzone HD: *Sanofi's Fluzone High-Dose Vaccine.*

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

Unless specified otherwise, the study population analyses will be based on the enrolled set.

6.1.1. Screening Failure

A summary of the number and percentage of participants who failed screening will be provided along with the inclusion/exclusion criteria failed. This summary will be based on the Screened Set.

6.1.2. Participant Disposition

A summary of the number and percentage of participants who completed the study as well as those who prematurely withdrew from the study will be provided. Reasons for withdrawal will be summarized for the Exposed Set.

The number and percentage of participant disposition and participants included in the Exposed Set and PPS will be summarized from Enrolled Set to Exposed Set and from Exposed Set to PPS respectively.

6.1.3. Demographic and Baseline Characteristics

The demographic characteristics including age, sex, ethnicity, height/weight, body mass index (BMI) (kg/m^2) on Day 1, race, country, and flu vaccination status in the past 2 years will be summarized with descriptive statistics. In addition, the following age categories will be summarized: 18-64, 65-84 and ≥85 based on the Enrolled Set, Exposed Set, and PPS.

Medical history will be tabulated by group and by MedDRA PT and SOC and listed using the Exposed Set.

6.1.4. Protocol Deviations

Important protocol deviations are a subset of protocol deviations (PDs) that may significantly impact the completeness, accuracy, or reliability of the study data or that may significantly affect a participant's rights, safety, or well-being. Important PD rules will be developed and finalized before database lock.

Important PDs include, but are not limited to, the following:

All participant data is excluded from the PPS:

- Study intervention not administered at all
- Invalid/missing informed consent
- Fraudulent data
- Participants got dosed but not as per protocol
- Study intervention storage temperature deviation which is not accepted by quality
- Expired study intervention administered
- Ineligible participant
- Participants randomized using the wrong stratification factor (incorrect age group, country, and/or flu vaccination history in preceding 2 years)

Participant data collected on the day or after the event is excluded from the PPS:

- Influenza disease infection, new malignancy and immunocompromised condition
- Administration of concomitant vaccine(s) forbidden in the protocol
- Administration of any medication forbidden by the protocol, namely administration during the study period of investigational or non-registered product (drug, vaccine or invasive medical device) other than the study intervention, of long-acting immune-modifying drugs or of immunoglobulins and/or any blood products or plasma derivatives.

Participant lab data collected on the day of the event is excluded from the PPS:

- Participants did not comply with blood sample schedule (see Table 9)

- Anti-CCI immunogenicity results not available pre- and post-dose
- Serological results available but results unreliable (e.g., wrong blood sample management)

The number of participants with important protocol deviations will be summarized by study group across time points and at Day 28 and Day 183. Important protocol deviations will be listed with date of occurrence, deviation description, and analysis set from which participant is excluded. Important protocol deviation summary will be based on the Enrolled Set.

The important PDs will be reported in the Clinical Study Report (CSR). The important PDs leading to elimination from the PPS will be summarized for the Exposed Set and the important PDs leading to elimination from the Exposed Set will be summarized for the Enrolled Set.

Table 9 Intervals between study visits

Interval[a]	Planned visit interval	Allowed interval range
Visit 1[b] → Contact 1	7 days	7-10 days
Visit 1[b] → Visit 2	28 days	28-35 days
Visit 1[b] → Contact 2	91 days	77-105 days
Visit 1[b] → Visit 3[c]	182 days	180-210 days

[a] Interval is computed as the difference between 2 dates

[b] Visit 1 corresponds to the day of the study intervention administration.

[c] Visit 3 will be replaced by a Contact if participant has received the standard of care vaccination against seasonal flu after Visit 2 and prior to this visit and did not report any adverse events that would require physical examination on site.

6.1.5. Prior and Concomitant Medications

Concomitant medications are defined as any medications and vaccines (other than study intervention) taken after the study intervention administration. Any medication or vaccine (other than study intervention) started prior to the study intervention administration and continued after the study intervention administration will be considered a concomitant medication.

The antipyretic classification is derived from the following ATC code:

ATC Code
A03D, A03DA, A03DB, A03DC, A03EA
M01, M01A, M01AA, M01AC, M01AE, M01AG, M01AB, M01AH, M01AX, M03B, M03BA, M03BB, M03BB, M03BC, M03BX
N02BG, N02AC, N02AG, N02AX, N02B, N02BA, N02BB, N02BE
R05, R05D, R05X

The percentage of participants using concomitant medication (any medication, any antipyretic and any antipyretic taken prophylactically, respectively) during the 28-day follow up period (i.e., Day 1 - Day 28 post-dosing) will be summarized by group.

The percentage of participants using antipyretic and/or analgetic medication will be summarized by group.

This antipyretic list may be revised based on WHO dictionary version.

Concomitant medications/vaccinations will be coded using both the GSK Drug and WHO Drug dictionaries and will be listed and summarized descriptively (any medication, any antipyretic, and any antipyretic taken prophylactically, respectively) by study group using the Exposed Set.

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

6.2.1. Endpoint Level Compliance

Solicited AE's

In terms of compliance for each of Day 1-7 and for each solicited symptom, the number/percentage of completed eDiary by either a participant or the investigator/study staff will be summarized study group and by dose, using frequency table. The denominator will be the number of expected completion day (i.e., the number of participants). For compliance of each day beyond Day 7, the number/percentage of completed eDiary by either a participant or the investigator/study staff will be summarized study group by dose, using frequency table. In this summary the denominator will be the number of expected completion day (i.e., number participants with the symptom on the previous day) and the numerator will be the number of participants with the symptom data among the participants contributing to the denominator.

An overall compliance summary will also be provided across symptoms and days in which the numerator and denominator will be the sum of numerators and denominator respectively of the previously described daily and symptom compliance specific summaries. The target compliance for the study is at least 80%.

The number/percentage of daily recordings from the investigator/study staff due to missed entries or due to erroneous participant entries will be summarized by study group using frequency table. In these summaries, the denominator will be the sum of all entries by either the participants or investigator).

The reasons for investigator/study staff entry will be summarized in frequency table.

6.3. Appendix 3 Data Derivations Rule

6.3.1. Data Derivation

6.3.1.1. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

6.3.1.2. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

6.3.1.3. Body Mass Index (BMI)

BMI will be calculated as follows:

BMI = (Weight in kilograms) / (Height in meters)^2

6.3.1.4. Temperature

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

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

6.3.1.5. Numerical serology results

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

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

6.3.1.6. Geometric Mean Titres (GMTs)

Geometric Mean Titre (GMT) calculations are performed by taking the inverse logarithm of the mean of the log titre transformations. Non quantifiable titres will be converted as described in Section 6.3.1.5 for the purpose of GMT calculation. Cut-off values are defined by the laboratory before the analysis.

6.3.1.7. Onset Day

The onset day for an event (e.g., AE, concomitant medication/vaccination) is the number of days between the study dose administration and the start date of the event. This is 1 for an event occurring on the same day as a study dose (and reported as starting after study dose).

6.3.1.8. Study Day

Study day will be calculated as follows:

- Study day prior to dosing will be calculated as: date of assessment – date of the study intervention administration
- Study day on or after the date of the dosing will be calculated as: date of assessment – date of the study intervention administration + 1.

6.3.1.9. Duration of Events

The duration of an event with a start and end date will be the difference between the start and end date plus 1 day, i.e., an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

For any grade, duration of solicited AE is defined as Start date – Stop date + 1, with Start date defined as the first day with the symptom at Grade > 0 within the 7-day solicitation period and Stop date defined as the last day with the symptom (i.e., Grade > 0) in or beyond the solicited period. For unresolved solicited AE continuing beyond Day 28 until end of study, stop date will be assigned the end of study date.

6.3.1.10. Counting Rules for Occurrences of Solicited and unsolicited Events

When the occurrences of solicited events are summarized, each event recorded as having occurred during a specific period will be counted as only 1 occurrence regardless of the number of days on which it occurs.

Accordingly for the summary presenting the number and percentage of participants reporting any solicited and unsolicited adverse events excluding SAE, a solicited AE starting within the solicited period (day 1-7) will be counted as one event while unsolicited AE, excluding prolonged solicited AE, will be counted as different event when these are associated to different MedDRA Primary Term or different start date. For instance, a participant with fatigue for several days within the solicited period, with fatigue prolonged beyond the solicited period and with fatigue restarting later in the

unsolicited period will contribute as 2 occurrence of fatigue. If a participant with fatigue for several days within the solicited period and extends beyond the solicited period, then this will contribute as 1 occurrence of fatigue.

6.3.1.11. AESIs

GSK MedDRA queries will be used to identify AESI:

- pIMD: refer to Table 11 of the protocol. AEs recorded and reported as pIMDs that are not listed in Table 11 of the protocol will be identified as pIMDs.
- Severe hypersensitivity (including anaphylaxis): Grade 3 unsolicited AEs under MedDRA SMQ hypersensitivity, narrow search (includes anaphylaxis), with an onset within 24 hours after dosing.
- Myocarditis/pericarditis. In addition to identification based on the medical and scientific judgement of the investigator, the following non-exhaustive list of PTs will be used: autoimmune myocarditis; eosinophilic myocarditis; giant cell myocarditis; hypersensitivity myocarditis; immune-mediated myocarditis; myocarditis; autoimmune pericarditis, pericarditis; pericarditis adhesive; pericarditis constrictive; pleuropericarditis.

These pIMD, myocarditis and pericarditis queries may be revised based on MedDRA dictionary version.

AESI summaries will include AEs identified by either the investigator or the MedDRA queries.

6.3.2. Handling of Partial Dates

6.3.2.1. Dates

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td>General</td><td>• When partially completed dates (i.e., dates missing a day and/or month) are used in calculations, the following standard rules will be applied:
o A missing day will be replaced by 15
o A missing day and month will be replaced by June 30th.
o For stop date, the maximum between the start and imputed stop date by above rule will be used instead.</td></tr>
<tr><td>Adverse Events</td><td>• Partial dates for AE recorded in the CRF will be imputed using the following conventions:
<table>
<tr><td>Missing start day</td><td>• If the event starts in the same month as the study dose administration then the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after study dose) will be used to complete the date. If ‘after study dose’ is selected, the imputed start date will match the study dose given during that month. If ‘before study dose’ is selected, the imputed date will be one day before the study dose given during that month.</td></tr>
<tr><td>Missing start day and month</td><td>• If the event starts in the same year as the study doses, the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after study dose) will be used to complete</td></tr>
</table></td></tr>
</table>

Element	Reporting Detail	
		the date. If 'after study dose' is selected, the imputed start date will match the study dose given during that year. If 'before study dose' is selected, the imputed date will be one day before the study dose given during that year.
	Missing end day	• the last day of the month or the last contact date will be used, which ever come first.
	Missing end day and month	• the last day of December or the last contact date will be used, which ever come first.
	Completely missing start/end date	• the last contact date will be used.
	• If the imputed end date is before the non-missing start date, then set the imputed end date as the start date • If the imputed start date is after a non-missing end date, then set the imputed start date as the end date	

All other cases of incomplete AE or concomitant medication/vaccination start date will follow the standard rules above.

6.3.2.2. Daily Recording of Solicited Events

Using electronic diaries for the collection of solicited events, a solicited event will be considered present only when a daily recording of Grade 1 or more is present. To determine the grading, the following rule will be used:

Administration Site (Local) reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Mild: Any pain neither interfering with nor preventing normal everyday activities.	Moderate: Painful when limb is moved and interferes with everyday activities.	Significant pain at rest. Prevents normal everyday activities.
Redness *	25 – 50 mm	51 – 100 mm	>100 mm
Swelling **	25 – 50 mm	51 – 100 mm	> 100 mm
Lymphadenopathy***	Present but does not interfere with activity	Interferes with normal activity	Prevents normal activity

* For redness and swelling, in addition to grading the measured administration site reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
*** Defined as localized axillary, cervical or supraclavicular swelling or tenderness ipsilateral to the administration arm.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	>38.9 >102.0
Headache	Headache that does not interfere with activity	Headache that interferes with normal activity	Headache that prevents normal activity
Fatigue	Fatigue that does not interfere with activity	Fatigue that interferes with normal activity	Fatigue that prevents normal activity
Myalgia	Myalgia present but does not interfere with activity	Myalgia that interferes with normal activity	Myalgia that prevents normal activity
Arthralgia	Arthralgia present but does not interfere with activity	Arthralgia that interferes with normal activity	Arthralgia that prevents normal activity
Chills	Chills present but do not interfere with activity	Chills that interfere with normal activity	Chills that prevent normal activity

6.3.2.3. Unsolicited Adverse Events

Unsolicited adverse event summaries are including serious adverse events unless specified otherwise.

For the summary of unsolicited adverse events, ongoing solicited events reported as unsolicited AEs will not be counted.

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

6.3.2.4. Laboratory Data

Biochemistry laboratory data requiring grading as per FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials the laboratory results (see table below) may have more decimals than expected.

To determine the grading, the following rule will be used:

1. The result expressed in the expected unit will be rounded to the number of decimals used for the grading (e.g., 1 decimal will be used for Creatinine and therefore 1.91 will be rounded to 1.9 and Grade as 2).

2. For grading defined from normal range such as liver function, the original results will be divided by ULN before applying rounding to the number of decimals used for the grading.

Table 10 FDA toxicity grading scales for biochemistry parameters evaluated in the current study

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN

ULN is the upper limit of the normal range.

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

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as potentially life-threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.

6.3.3. Display of Decimals

6.3.3.1. Percentages

Percentages and their corresponding confidence limits will be displayed with 1 decimal except for 100%, in which case no decimal will be displayed.

6.3.3.2. Differences in Percentages

Differences in percentages and their corresponding confidence limits will be displayed with 2 decimals.

6.3.3.3. Demographic/Baseline Characteristics Statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, BMI, pre-dose body temperature) will be presented with 1 decimal.

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

The maxima and minima of transformed height/weight variables will be displayed without decimals.

The maximum and minimum of transformed body temperatures will be displayed with 1 decimal.

6.3.3.4. Serological Summary Statistics

The number of decimals used when displaying GMT and their confidence limits is assay specific based on the magnitude of the assay result post-dose and the clinically relevant assay threshold. The same number of decimals will be used for a given assay regardless of the timepoint presented.

GMT fold increase from pre-dose follows the same principle. Namely, when the lowest clinically relevant threshold is 2-fold, 2 decimals are displayed while when the lowest clinically relevant threshold is 4-fold, 1 decimal is displayed.

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

6.3.4. Statistical Methodology

6.3.4.1. Exact Confidence Intervals around Proportions

The exact confidence intervals around within-group proportions are derived using the method of Clopper and Pearson [Clopper, 1934].

6.3.4.2. Standardized Asymptotic Confidence Intervals Around Differences in Proportions

The standardized asymptotic confidence intervals around differences in proportions are derived using the method of Miettinen and Nurminen [Miettinen, 1985]

6.3.5. Trademarks

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