STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 1 Randomized, Observer Blind, Placebo Controlled, Dosage-Escalation Single Center Study to Evaluate the Safety and Immunogenicity of an RSV Fusion Glycoprotein (F) Subunit Vaccine in Healthy Adults
Study Number:	V122_01
Protocol Version and Date:	3.0, 26 AUG 14
Phase of Development:	Phase 1
Sponsor:	Novartis Vaccines
Plan Prepared by:	PPD
Version and Date:	Version 1.0: 09 DEC 14
Approvers:	PPD, Supervisory Biostatistician
	PPD, Cluster Head
	PPD, Cluster Physician
	PPD, Clinical Trial Leader
	PPD, Medical Writer

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CSR	Clinical Study Report
CTL	Clinical Trial Leader
DMC	Data Monitoring Committee
FAS	Full Analysis Set
MedDRA	Medical Dictionary for Regulatory Activities
PD	Protocol Deviation
PPS	Per Protocol Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Content

1. BACKGROUND AND RATIONALE

Respiratory syncytial virus (RSV) is the most important cause of acute lower respiratory tract infections (ALRIs) that result in hospital visits during infancy and early childhood. In the United States, more than 60% of infants are infected by RSV during their first RSV season, and nearly all have been infected by two to three years of age. Disease burden is similar in Europe; globally, among children less than five years of age, RSV causes an estimated 33.8 million ALRIs each year (more than 22% of all ALRIs), resulting in 66,000-199,000 deaths, 99% of which occur in developing countries.

In addition, RSV is a common cause of respiratory disease among the elderly, resulting in as many hospitalizations as influenza in a heavily influenza-immunized population.

Novartis Vaccines and Diagnostics (NVD) has developed an investigational RSV subunit vaccine from an engineered recombinant RSV fusion (F) glycoprotein. The clinical program will evaluate the feasibility of passively protecting infants by immunizing pregnant women with the RSV F subunit vaccine. If maternal immunization during 24 to 32 weeks of gestation increases antibody titers eight-fold, the median peak of RSV disease in infants would be delayed from its current peak at two to three months of age to a new peak at approximately five to six months of age. This delay could significantly decrease the burden of RSV disease in infants in the first months of life and open an RSV disease-free interval during which active immunization of infants could further extend protection beyond six months of age. In the current study, we will test one dosage of the investigational RSV F subunit vaccine that is presumed to be below the dosage-response plateau (to establish that a maximal neutralizing antibody response cannot be achieved with a low dosage) and two higher dosages (to either establish the dosage-response plateau or indicate that higher dosages are needed in a subsequent study to reach the plateau).

The purpose of this study is to evaluate the safety and immunogenicity of two doses of the investigational RSV F subunit vaccine administered intramuscularly (IM). In this current Phase 1, first-in-human study, the three different antigen amounts that have been selected will be evaluated in a stepwise manner in three different cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 µg], cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and cohort 3: high dosage of RSV F subunit vaccine [135 µg]). In addition, the effect of an adjuvant, either aluminum hydroxide or MF59, and antibody kinetics post-vaccination at different time points will be evaluated as compared to unadjuvanted RSV F subunit vaccine at the same dosage levels.

For further details please refer to section 1.0 of the protocol.

2. OBJECTIVES

2.1 Primary Objective(s)

Primary Immunogenicity Objective

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Safety Objective

1. To assess the safety of the RSV F subunit vaccine compared to placebo.

2.2 Secondary Objectives

Secondary Immunogenicity Objectives

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).
2. To evaluate the total serum antibody responses to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
3. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to the RSV protein F in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
4. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to each of the RSV proteins G and N in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

Secondary Exploratory Objectives

1. To characterize the serum neutralizing antibody (NAb) and binding antibody responses against additional RSV strains and native or engineered RSV antigens at baseline and following vaccination in a subset of subjects.
2. To determine the frequency of B cells specific for RSV proteins in a subset of subjects, and subsequently, explore the baseline immunity to RSV and the immune response to the RSV F subunit vaccine by analyzing the RSV-specific B-cell repertoire in a selected group of the subset of subjects.

3. STUDY DESIGN

This is a Phase 1, randomized, observer blind, placebo-controlled, dosage-escalation, single center study, enrolling healthy adults. In total, approximately 288 healthy non-pregnant female and male adults (18 to 45 years of age) will be enrolled in the study in the ratio of 3:1. There is a higher ratio of female to male subjects because the RSV F subunit vaccine is ultimately intended for use in pregnant women. Men are still being included in this study because the vaccine may also be tested in the future in elderly subjects.

Approximately 288 healthy subjects will be enrolled (1:1:1) in a stepwise dosage-escalation manner in to one of three cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 μg], cohort 2: middle dosage of RSV F subunit vaccine [90 μg], and cohort 3: high dosage of RSV F subunit vaccine [135 μg]). Cohort 1 will be enrolled first, followed by cohort 2, and finally cohort 3.Within each cohort subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or saline placebo, as outlined in Table 1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine, with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group and 24 placebo subjects per cohort) (Table 1).

Table 3-1: Times and Events Table

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Informed Consent	X														
Medical History	X														
Physical Exam [a]	X	X [b]		X		X [b]		X		X	X	X	X	X	
Safety Laboratory Blood draw [max: 10 mL whole blood] [c]	X	X [b]		X		X [b]		X		X					
Urinalysis [d]	X	X [b]		X		X [b]		X		X					
Pregnancy Test	X	X [b]				X [b]									
Exclusion/Inclusion Criteria	X														
Randomization		X [b]													
Serology Blood draw [max: 15 mL whole blood] [e]		X [b]				X [b]				X			X		

Study Period	**Screen**	**Vaccine Dose #1 & Safety Follow-up**				**Vaccine Dose #2 & Safety Follow-up**					**Post-vaccination Follow-up**				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
2° Exploratory Obj. Blood Draw [max: 50 mL whole blood] [f]		X [b]		X		X [b]							X		
Study Vaccine Administered [g]		V				V									
30 Minutes Post Injection Assessment (Local/ Systemic AEs, Body Temperature) [h]		X				X									
Local/Systemic AEs, Body Temperature, Other Indicators of Reactogenicity [i]		X				X									
Diary Card Training [j]		X				X									
Diary Card Dispensed [k]		X				X									
Diary Card Reminder Call [l]			X				X								

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Diary Card Reviewed and Collected [m]				X				X							
Telephone Contact for Review of Safety Data [n]					X				X						
Assess all AEs, including SAEs and NOCDs Leading or not to Study Withdrawal [o]		X		X		X		X		X	X	X	X	X	X
Assess for AESIs [p]		X		X		X		X		X	X	X	X	X	X
Prior/Concomitant Medications/vaccines [q]	X	X		X		X		X		X	X	X	X	X	
Study Termination [r]															X

a. Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log. See section 6.2 for components of physical examination by visit.
b. Procedure to be performed prior to vaccination.
c. Safety laboratory assays that will be included are listed in section 3.5.3.
d. Urinalysis testing will include at a minimum protein, glucose, and presence of red blood cells.

e. Maximal blood draw refers to volume drawn for each type of assay at each specified visit. See section 3.5.1 for greater detail regarding blood sampling volumes.
f. The first 10 subjects enrolled in each treatment group of Cohort 3 who agree to the additional blood draws will need to sign an additional consent prior to the blood draw for the secondary exploratory objective.
g. Subjects will receive two doses of vaccine or placebo according to the study randomization scheme.
h. A 30 minute post-injection local and systemic adverse event and body temperature measurement will be performed by the subject under site staff supervision at the clinic during Visit 1 and Visit 5.
i. Beginning 6 hours following study vaccine administration at Visit 1 and Visit 5, and daily thereafter through 7 days after each vaccination, solicited local and systemic adverse events including other reactions (i.e. body temperature measurements and use of analgesics/antipyretics) will be reported daily by the subject on a diary card.
j. Subjects will receive training on the diary card at Visit 1 and Visit 5.
k. The diary card will be dispensed at Visit 1 and Visit 5, and subjects will be reminded that diary cards must be returned at the next clinic visit (Visit 2 or Visit 6).
l. Site staff will contact subjects by phone 2 and 4 days after each vaccination to remind them to complete their diary cards each day, and to bring the diary cards to their next clinic visit.
m. Review of safety data captured on diary cards will be completed at Visit 2 and Visit 6. Diary cards will be collected and stored with subject files.
n. Safety data will be collected for 28 days following each vaccination. At 14 and 21 days after each dose, subjects will be interviewed by site staff using a scripted interview for collection of safety data. These safety data will be transcribed on source documents by the site staff performing the interviews.
o. All medically attended AEs that lead to an unscheduled visit to a healthcare practitioner and/or a visit to the emergency department or its equivalent will be collected for 28 days following signing of the informed consent. SAEs, NOCDs and AEs leading to study or vaccine withdrawal will be collected through 1 year after receipt of the second dose. Please see section 6.6 for greater detail regarding methods for SAE and AEs leading to study or vaccine withdrawal collection.
p. Adverse events of special interest (AESIs) will also be documented in all study subjects for the duration of the study. Monitoring for AESIs will be extended through 1 year after receipt of the second dose and will be accomplished at clinic visits or by a telephone follow-up call. A tabulation of all AESIs, categorized by MedDRA preferred terms and assessed relationship to study vaccine will be submitted as an addendum to the Clinical Study Report (CSR) if not included in the CSR.
q. Collect concomitant medications and vaccination history according to the study procedures outlined in protocol section 3.2.5 and 5.4.
r. Any subject who terminates the study after receipt of vaccine (prior to Visit 9) is recommended to undergo study-related procedures required at Visit 9. For subjects who terminate after Visit 9, a telephone contact to assess for SAEs/AEs and associated concomitant medications is required.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Subjects will be enrolled in a stepwise dosage-escalation manner into one of three cohorts:

- Cohort 1: low dosage of RSV F subunit vaccine [45 µg],
- Cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and
- Cohort 3: high dosage of RSV F subunit vaccine [135 µg]).

Within each cohort, subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or placebo, as outlined in Table 4.1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group, and 24 placebo subjects in each cohort.

Table 4.1.

Cohort	Dosage	Route of Administration	Regimen: Two Doses †		
			No Adjuvant	Al(OH)3 ‡	MF59 ‡
1	45 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
2	90 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
3	135 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		

For further details please refer to section 5.1. of the protocol.

4.1.1 Definition of Vaccination Errors

The list below provides some examples of potential errors that may occur during vaccination:

- Subjects got vaccinated with a vaccine different from the one assigned at randomization

- Subjects got vaccinated with the correct vaccine but containing a lower volume
- Subjects got vaccinated with different vaccine at the second dose.

Please see Section 7 a complete guidance on how vaccination errors are handled in the statistical analysis.

4.2 Blinding and Unblinding

For details please refer to section 3.3 of the protocol.

If a subject is unblinded during the study, it is to be reported as a major protocol deviation, except for Pharmacovigilance unblinded suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately (for details see SOP BCDM-04).

The first-line analysis excludes unblinded subject(s) in immunogenicity or efficacy statistical analyses based on the per-protocol set. The unblinded subjects will be included in the full analysis set (FAS) and safety sets.

Further details on measures taken to ensure blinding can be found in the study-specific Data Security Plan.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

For details please refer to section 7.4.2.4 of the protocol.

Sample size/power considerations are included in the study protocol. Technical details including statistical assumptions and software are given in a separate sample size memo authored by the study Biostatistician. In the same document, a statistical co-reviewer verified and documented the sample size/power considerations. This document was completed prior to finalization of the protocol and stored in "Entreprise/eTMF Repository/V122_01/Cluster Documents/Statistical analysis /Statistical Analysis Plan/Other".

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

Clinical Study Report (CSR) reportable protocol deviations (PDs) are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial.

All reportable PDs will be evaluated before unblinding and classified according to ICH into the following five categories:

- Subject developed withdrawal criteria during the study but was not withdrawn
  - Underlying medical condition forbidden by the protocol or which may influence immune response.
  - Subject had contraindication for a subsequent study vaccination but was vaccinated.
  - Concomitant infection related to the vaccine which may influence immune response.
- Subject received wrong vaccine or incorrect dose
  - Study vaccine was not administered at all
  - Vaccine administration not according to protocol.
  - Randomization failure.
- Subject took an excluded concomitant medication
  - Administration of concomitant vaccine(s) forbidden in the protocol.
  - Administration of any medication forbidden by the protocol.
- Subject randomized and did not satisfy the entry criteria
  - Subject did not meet entry criteria.
- Key study procedures missed or performed out of window
  - Randomization code was broken.
  - Subject did not comply with study vaccination schedule.
  - Subject did not provide any post-vaccination safety data.
  - Subject did not comply with blood draw schedule.
  - Serological results not available post-vaccination.

- Obvious incoherence or error in data.

CSR reportable PD will lead to exclusion of the subject or part of the subject's data from at least one analysis set.

The number of subjects in any and by PD category will be summarized by vaccine and overall and individual subject listings will be provided in an appendix.

Prior to unblinding, designated Novartis Vaccines staff will develop a memo that describes the PDs that lead to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Cluster Physician and will be included in the trial master file (Exclusion Memo).

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of adverse events (including death) is not considered as a PD. The missing assessments that should have otherwise been collected for that subject later in the study are also not considered as a PD.

Comments:

- Missed safety calls are not considered reportable protocol deviations.
- Implausible measurements do not lead to PD.

6.2 Determination of Protocol Deviations

Prior to unblinding, a set of listings will be provided to the Cluster Physician and the Clinical Trial Leader (CTL) for review according to SOP MON-11.

The listings will be programmed following the list presented in table in section 7.3.8, and specifically using the PD codes specified in the first column.

After the review, the Cluster Physician and the CTL will provide the Biostatistician with:

- An assessment of CSR reportable PD based on blinded clinical data review.
- An assessment of subjects without PD (e.g., premature withdrawals due to adverse event, withdrawal of consent) who should be excluded from an analysis set.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible. Therefore these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	Values ≥ 900 mm Measurements < 0 mm
Induration	Values ≥ 500 mm Measurements < 0 mm
Swelling	Values ≥ 500 mm Measurements < 0 mm

7. ANALYSIS SETS

7.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the trial, and receive a subject ID.

Demography and baseline characteristics tables as well as subject listings will be produced on the All Enrolled Set.

7.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3 Full Analysis Set (FAS) Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (FAS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (FAS 1-9).

In case of vaccination error, subjects in the FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

If a subject is unblinded during the study, he/she will be included in the FAS.

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points).
- Have no major protocol deviations leading to exclusion (see section 6.2) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see section 6.2)

In detail:

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (PPS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (PPS 1-9).

For secondary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 29 (PPS 5).
- Provide immunogenicity data for anti-RSV NAb at day 181 (PPS 12).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 29 (PPS 1-5).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 181 (PPS 1-12).
- Provide immunogenicity data for RSV proteins F, G and N at baseline (PPS 1)
- Provide immunogenicity data for RSV proteins F, G and N at day 29 (PPS 5)
- Provide immunogenicity data for RSV proteins F, G and N at day 57 (PPS 9)
- Provide immunogenicity data for RSV proteins F, G and N at day 181 (PPS 12)

In case of vaccination error, the subject is excluded from the PPS. If a subject receives a vaccine, labelled for another subject but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

A minimum of 4-fold increase in antibody titers against RSV N or G from baseline to post vaccination will be considered indicative of possible natural RSV infection. This will be considered a CSR-reportable protocol deviation leading to exclusion from the PPS with associated code 250 “Concomitant infection which may influence vaccine-specific immune responses”.

If a subject is unblinded during the study, except for SUSAR, he/she will be excluded from all PPS analyses.

7.5 Safety Set

Solicited Safety Set

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

For the by vaccination tabulations, solicited safety sets will be defined as follows:

- Solicited safety set #1 for post-injection reactions after the first dose (SOL 1)
- Solicited safety set #2 for post-injection reactions after the second dose (SOL 2)

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited adverse event data.

In this respect, a confirmation of no AE is considered as adverse event data; hence subject is to be included.

Three Unsolicited Safety Sets will be defined:

- Unsolicited Safety Set Day 1 – Day 57 (UNSOL 1-57)
- Unsolicited Safety Set Day 58 – Day 394 (UNSOL 58-394)
- Unsolicited Safety Set Day 1 – Day 394 (UNSOL 1-394)

Overall Safety Set

All subjects who are in the solicited safety set and/or in the unsolicited safety set.

Subjects providing only 30 minutes post-vaccination safety data will be reported separately in a 30 minute post-vaccination safety analysis and excluded from all other safety analysis.

In case of vaccination error, subjects will be analyzed as “treated” (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.5.1 Restricted Safety Set

Not applicable

7.6 Other Analysis Set

Not applicable

7.7 Overview of Analysis Sets by PD Code

Table 7.7-1: Safety Sets

PD code	PD Description	Study Objective/ Period	All Exposed Set	Overall Safety Set	Safety Set, Unsolicited AEs	Safety Set, Solicited AEs, Period (i), T6H-D7	Safety Set, Solicited AEs, Period (i), T6H-D3	Safety Set, Solicited AEs, Period (i), D4-D7	Safety Set, Solicited AEs, Period (i), T30m
	Exclusion code		**EXPFL**	**SAFFL**	**SSU10FL**	**SSS10FL**	**SSS11FL**	**SSS12FL**	
100	**Study vaccine not administered AT ALL**	All Study	EXC	EXC	EXC	EXC	EXC	EXC	EXC
115	**Subject did not provide any post-vaccination unsolicited safety data**	All Study			EXC				
116	**Subject did not provide any post-vaccination solicited safety data**	Period (i)				EXC	EXC	EXC	EXC

EXC = excluded from this analysis set.

Table 7.7-2: Immunogenicity Sets

PD code	PD Description	Study Objective / Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x
	Exclusion code		**FAS1FL**	**FASxFL**	**FAS1xFL**	**PPS1FL**	**PPSxFL**	**PPS1xFL**
100	**Study vaccine not administered AT ALL**	All Study	EXC	EXC	EXC	EXC	EXC	EXC
110	**Serological results are not available**	Visit 1 (baseline)	EXC		EXC	EXC		EXC
110	**Serological results are not available**	Visit x		EXC	EXC		EXC	EXC
112	**Obvious deviation from Laboratory Manual or error in laboratory data**	All Study				EXC	EXC	EXC
120	**Randomization failure**	All Study				EXC	EXC	EXC
120.2	**Subject received another vaccine than allocated (Actual Arm different from Planned Arm)**	All Study				EXC	EXC	EXC
130	**Randomization code was broken**	All Study				EXC	EXC	EXC
140	**Vaccination not according to protocol**					EXC	EXC	EXC
140.4	**Incomplete vaccination series (only 1st dose administered)**	All Study					EXC*	EXC
150	**Administration of forbidden vaccine**							
200	**Subject did not meet entry criteria**	All Study				EXC	EXC	EXC
220	**Subject had contraindication for a subsequent study vaccination but was vaccinated**	Visit 5				EXC	EXC	EXC
230	**Administration of forbidden medication**					EXC	EXC	EXC
240	**Underlying medical condition forbidden by the protocol**					EXC	EXC	EXC
250	**Concomitant infection related to the vaccine which may influence immune response**					EXC	EXC	EXC
260	**Did not comply with study vaccination schedule**					EXC	EXC	EXC

PD code	PD Description	Study Objective / Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x
	Exclusion code		**FAS1FL**	**FASxFL**	**FAS1xFL**	**PPS1FL**	**PPSxFL**	**PPS1xFL**
270	**Did not comply with blood draw schedule**					EXC	EXC	EXC
280	**Subject who developed withdrawal criteria but was not withdrawn**	Visit x					EXC	EXC
290	**Early withdrawal**	Visit x		EXC	EXC		EXC	EXC

*except from PPS 5 as deviation would occur after blood draw for day 29 assessment.
FAS = Full Analysis Set; PPS=Per Protocol Set; EXC = excluded from this analysis set.
FAS 1 and PPS 1 refer to any immunogenicity analyses including values for baseline.
FAS x and PPS x refer to any immunogenicity analyses including values for visit x (i.e. visits after V1 with immunogenicity evaluation).
FAS 1x and PPS 1x refer to any immunogenicity analyses including values for baseline and visit x.
Code 999 is not a protocol deviation but it will lead to exclusion.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The log-transformed antibody titers at the time points where a blood draw for immunogenicity is collected will be analyzed using an Analysis of Covariance (ANCOVA) model which includes the vaccine-group effect (see section 11.2) and an effect defined by the log-transformed pre-vaccination antibody titer as an independent variable. Summary tables will show adjusted geometric mean titers (GMTs) for each vaccine group.

Binary data tables will show unadjusted percentages.

8.2 Handling of Dropouts, Missing Data

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: “day 1 – day 28, day 29 – day 57, day 58 – day 394”.

For solicited adverse events, the solicited study period “30 min - day 7” will be divided into: “30 min, 6h - day 3, day 4 - day 7, and 6h - day 7”.

No imputation methods will be used to address missing values.

8.2.2 Immunogenicity Data

Missing immunogenicity values are considered MCAR and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used.

Population of analyses:

PPS will be considered the principal analysis set. The primary immunogenicity analyses will be conducted on the PPS and will be repeated using the FAS as a measure of robustness. All other immunogenicity analyses will be based on the PPS.

8.2.3 Efficacy Data

Not applicable.

8.3 Multicenter Studies

Not applicable as this is a single center study.

8.4 Multiple Comparisons and Multiplicity

Statistical tests will only be used for descriptive purposes therefore no multiplicity adjustment will be done.

8.5 Immunogenicity/Safety/Other Subsets

Not applicable.

8.6 Subgroups

Not applicable.

8.7 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

(Date of Visit 1 – Date of Birth + 1) / 365.25

Body Mass Index (kg/m^2) will be calculated using the following formula:

Weight (kg) / Height^2 (m^2)

Immunogenicity

Values below the limit of quantification (LQ = 1:100, recorded as "< LQ") will be set to half that limit (i.e., LQ/2 = 1:50).

Geometric Mean Titer

The GMT will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(t_i)}{n}\right\}}$$

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers/concentrations.

Duration in the Study

Duration in the study is defined in days as:

Last visit date (visit x)[a] – Enrollment date (visit 1) + 1

[a]or premature discontinuation date (in case of withdrawal from the study)

The duration is missing if one of the dates is missing or incomplete.

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

All adverse events will be characterized according to the date of occurrence related to the vaccination phase as follows:

- **Emergence before vaccination phase**: start date before the first date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the first date of injection of study vaccine or, adverse event increase in severity including to "serious" adverse event.

If start date is equal to the first date of injection then "timing" variable ("On injection day, before injection"/"On injection day, after injection") will be used to define whether the adverse event occur before or after the injection.

If there are several vaccinations, the adverse event will be associated with the most recent vaccination.

If an adverse event start date is missing or unknown, the adverse event will be considered as emergent.

When start and/or end dates of an adverse event are only partially known, adverse events will be categorized as emergent before, during, or after vaccination phase using the following rules:

- If the partial end date is before (<) the first study vaccination (i.e., year or year & month is/are before the first study vaccination year or year & month) then the adverse event is emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the adverse event is emergent during vaccination phase.

The **maximum event severity** is the greatest severity associated with a preferred term (PT) for a reported adverse event according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe (except for the definition of emergence).

Multiple AEs with the same PT for the same subject are counted only once.

Vaccination-related Adverse Events are those for which the relationship has been evaluated by the investigator, and recorded either as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

All laboratory measurements summarized in the CSR are those defined in the study protocol. All laboratory tests including retests and reasons for retests will be presented in the data listings for each subject. In case of multiple measurements of a laboratory parameter for a subject:

1. Before the study vaccination, only the latest measurement, with closest date before (<) the first study vaccination date, will be retained for analysis.
2. After the study vaccination, only the first measurement, with soonest date after (>) the last study vaccination date, will be retained for analysis.

Reference ranges used to categorize the results as "low" (values below the lower limit of the reference range), "normal" (values within the reference range) or "high" (values above the upper value of the reference range) will be those provided by Quintiles Laboratories, which performed the tests and provided the laboratory reports.

Prestudy, Concomitant and Post-Vaccination Medications

A **previous medication** is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date).

All other medications are **concomitant**.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule.

If the first study vaccination date is missing then the medication is considered as concomitant with the study vaccination schedule, provided that the study vaccine was administered to the subject.

For further details please refer to the technical and program specifications document stored in 'Home/analysis/V122/V122_01/final/prod/docs' within the SAS Drug Development (SDD) server.

8.8 Analysis Software

All analyses will be performed using SAS Software version 9.1 or higher.

8.9 Data Transformation

Distributions of antibodies are generally skewed to the right (Nauta, 2010). Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers will be log10-transformed. GMTs and their 95% CIs are computed by exponentiating (base 10) the least squares means and 95% CIs of the log10 titers.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All subjects who provide informed consent will be accounted for in this study. The frequencies and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will also be presented.

The time the subjects are under observation will be summarized by vaccine and overall using summary statistics (mean, SD, minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median and range, and will be calculated by vaccine group and overall.

The frequencies and percentages of subjects by sex, ethnic origin, race, entry criteria fulfilled will be presented by vaccine group and overall. Demographic data will be tabulated for the All Enrolled, FAS, PPS and Safety sets.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA S system organ class (SOC) and preferred term (PT), by vaccine group and overall. Medical history data will be tabulated for the All Enrolled Set.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled set.

11.2 Primary Objectives Analysis

Primary Objective:

To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Immunogenicity Endpoints:

- Geometric mean titer (GMT) of the serum anti-RSV neutralizing antibody(NAb) titer at Day 57 (28 days after the second dose).
- Proportion of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from Day 1 (baseline) to Day 57 (28 days after the second dose).

It is assumed that log-transformed antibody titers are normally distributed; therefore values will be logarithmically transformed (base10) and all statistical analyses on antibody levels will be performed on the logarithmic scale.

Statistical models:

GMTs:

For each treatment group, GMTs of the serum anti-RSV NAb along with their associated 95% confidence intervals (CIs) will be computed by exponentiation of the corresponding log-transformed means and 95% CIs. In addition, median, minimum and maximum values will be calculated for each vaccine group at each time-point.

Adjusted GMTs will come from an ANCOVA model including fixed factor for group and covariate adjustment for baseline value.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;
BY antigen visitnum;
class group;
model logtiter_post = group logtiter_pre;
LSMEANS group / stderr cl   OUT=LSMEANS;
```

```
RUN;
```

The factorial design of the study allows fitting a statistical model able to evaluate the impact of each factor in explaining the antibody response; the following analysis of covariance (ANCOVA) model will be utilized:

$$Y_{ijk} = \mu + a_i + \beta_j + \gamma X_{ijk} + E_{ijk}$$

where Y_{ijk} represents the log-transformed (base 10) titer of the k^{th} subject in the dosage group i (i= 45µg, 90 µg or 135 µg) and adjuvant group j (j= no adjuvant, aluminum or MF59); µ is the overall mean; a_i is the main effect of dosage i; β_j is the main effect of adjuvant j, γ is a parameter included to adjust for continuous covariate X_{ijk} representing baseline titer; E_{ijk} are error terms normally distributed with mean zero and variance σ^2.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;

BY antigen visitnum;

class dosage adjuvant;

model logtiter_post = dosage adjuvant logtiter_pre;

LSMEANS dosage adjuvant / stderr tdiff cl pdiff    OUT=LSMEANS;

ESTIMATE "dose 45 vs dose 90"  dose   1 -1  0;

ESTIMATE "dose 90 vs dose 135" dose   0  1 -1;

ESTIMATE "dose 45 vs dose 135"  dose   1  0 -1;

ESTIMATE "no adj vs alum" adjuvant 1 -1 0;

ESTIMATE "alum vs MF59" adjuvant 0 1 -1;

ESTIMATE "no adj vs MF59" adjuvant 1 0 -1;

RUN;
```

where `logtiter_post` represents the log-transformed antibody value of the immunogenicity variable at a given post baseline timepoint, `dosage` indicates the amount of vaccine content, `adjuvant` the type of adjuvant (i.e none, aluminium hydroxide or

MF59) , `logtiter_pre` the log-transformed antibody baseline value of the immunogenicity variable.

Placebo will not be considered in this analysis.

Analysis of interaction:

To test for a possible effect modifier, a dosage by adjuvant interaction will be added to the previous ANCOVA model. With an overall p-value smaller than 0.05, further investigations will be conducted to better understand the degree of the interaction. Additional examinations will include point estimates and 95% CIs for the different levels (e.g. low dosage by no adjuvant, low dosage by alum and so forth) of the factors under study.

4-fold increase:

Proportions of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from baseline will be presented, for each treatment group within each cohort, together with their two-sided 95% Clopper-Pearson CIs. Group differences will not be produced.

11.3 Secondary Objectives Analysis

The same statistical model as the one used for the primary objectives will be applied. No test for interaction will be conducted for the GMT analyses.

Reverse Cumulative Distribution Functions (RCDFs) will be produced.

11.4 Exploratory Objectives Analysis

Not Applicable as exploratory objectives will not be part of the core analysis of the present study. Data for the exploratory analyses will be released after the CSR for the study will be finalized.

A separate SAP to define the analyses for the exploratory objectives will be created at a later time.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not Applicable.

12.2 Secondary Objectives Analysis

Not Applicable.

12.3 Exploratory Objectives Analysis

Not Applicable.

13. SAFETY ANALYSIS

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure.
- Solicited local and systemic adverse events and indicators of solicited adverse events.
- Unsolicited adverse events.
- Clinical Laboratory Investigations.

13.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed diary cards, irrespective of severity. The analysis will show the number of subjects with *valid data* by solicited adverse event and time point. *Valid data* in the context of the safety completeness analysis are all data entered in the diary card (including implausible values) except "Not done/unknown".

Four summaries will be produced:

1. The frequencies of subjects who provide diary cards by vaccine group and collection method (i.e., clinical visit, postal mail).
2. For each solicited adverse event, the frequencies of subjects with *valid data* will be presented by vaccine group and time point: 30 min, 6h, days 2, 3, 4, 5, 6 and 7
3. For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7
4. For each solicited adverse event, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

All analyses will be based on the 'as treated' analysis set.

13.2 Solicited Local and Systemic Adverse Events

For details please refer to section 7.1.1 of the protocol.

Only solicited local and systemic adverse events reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix.

Solicited adverse events will be reported at 30 minutes, at approximately 6 hours post-vaccination on day 1 and then daily until day 7 using structured diaries. The analyses of solicited adverse events will be done separately for 30 minutes and based on three intervals: 6h - day 3, day 4 - 7 and 6h - day 7, each without 30 minutes data. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AEs.

For erythema, induration and swelling recorded originally as diameters (mm), the following categorization will be used to summarize the data:

Grade 0 (< 25 mm), any (25-50 mm, 51-100 mm, >100 mm)

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 3 schemes described below:

- by 0.5 °C increments: <36.0, ≥36.0-<36.5, ≥36.5-<37.0, ≥37.0-<37.5, ≥37.5-<38.0, ≥38.0-<38.5, ≥38.5-<39.0, ≥39.0-<39.5, ≥39.5-<40.0, ≥40.0°C
- by 1.0 °C increments: <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C
- <38.0, ≥38.0 °C

Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as an indicator of a systemic adverse event.

The analyses will encompass summaries of the data on five levels:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).
3. Solicited adverse events, maximum event severity by event and interval (6h - day 3, day 4 -7, and 6h - day 7, each without 30 min).
4. Duration of solicited adverse events.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval (6h-Day 3, Day 4-7 and 6h-Day 7, each without 30 min).

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

For each of the time points only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse event in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator in order to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 2: Time of first onset of solicited adverse events

The **time of first onset** is defined, for each subject, for each solicited adverse event, as the time point at which the respective solicited adverse event first occurred. For erythema, induration and swelling the following threshold will be used ≥ 25 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited adverse events by vaccine group, by vaccination number, and by each time point.

The tables below show examples of a listing considering four subjects who receive two vaccinations, with post-vaccination data for a given solicited adverse event and a summary table by vaccination group.

Table 13.2-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	6 Hours	Day 2	Day 3	Day 4	…	Day 7
1	PPD	None	Severe	Moderate	None	…	None
	PPD	Mild	None	None	Moderate	…	Missing
	PPD	Moderate	Mild	None	Severe	…	Mild
	PPD	Mild	Mild	None	None	…	None
2	PPD	None	None	None	None	…	Not done
	PPD	None	Mild	Mild	Missing	…	Missing
	PPD	Severe	None	Mild	Missing	…	None
	PPD	Missing	Missing	Missing	Severe	…	Mild

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'.

Table 13.2-2: Time to First Onset of Solicited Adverse Events

Vaccine group A

Vaccination	Adverse event		Number (%) of Subjects					
			6 HRS (N=4)	DAY 2 (N=4)	DAY 3 (N=4)	DAY 4 (N=4)	…	DAY 7 (N=4)
1	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	1 (25.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	0 (0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
2	XY	n	3	3	3	2	…	2
		ANY	1 (33.3%)	1 (33.3%)	0 (0%)	1 (50.0%)	…	0 (0%)
		Mild	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (33.3%)	0 (0%)	0 (0%)	1 (50.0%)	…	0 (0%)
ANY	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (25.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)

N: no. of subjects with data at a time point across all vaccinations.
n: no. of subjects with data at a time point for that specific vaccination.

Level 3: Solicited adverse events, maximum event severity by event and interval

The **maximum event severity** will be defined if there is at least one plausible non-missing observation (excluding "Not done/unknown" and implausible values) within this time interval. Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each adverse event, followed by a summary across subjects for each vaccine. Subjects without any solicited adverse events in the interval, i.e., missing values at each of the requested time points,

will be removed from the denominator. Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 4: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema, induration and swelling. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

The following example is used to illustrate how the duration is calculated:

Suppose six subjects, who received a vaccination have the post-vaccination solicited adverse event data shown in the table below. In addition, there are unsolicited adverse event reports indicating that the adverse event in subject PPD and PPD continued until day 12 and day 8, respectively. For subject PPD the number of days is calculated as 6+5 and for subject PPD as 3+1. Missing values ('Missing') are not taken into consideration

Table 13.2-3: Example for Number of Days With Solicited Adverse Events

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
PPD	None	Severe	Moderate	None	None	None	None	2
PPD	Mild	None	None	Moderate	Moderate	Moderate	Missing	4
PPD	Moderate	Mild	None	Severe	Severe	Severe	Mild[a]	11
PPD	None	None	None	None	None	None	Not done	0
PPD	None	Mild	Mild	Missing	Missing	Missing	Missing	2
PPD	Severe	None	Mild	None	None	None	Severe[b]	4

[a] continued until day 12; [b] continued until day 8

The frequency distribution of the number of days will be provided in a summary table by vaccine group, vaccination number, and by solicited adverse event.

Level 5: Solicited adverse events, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited adverse event** is defined as "any" for a subject if he/she reports greater than "none" ≥ 25 mm, for erythema, swelling, and induration) for the respective event and "none" otherwise. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic,

any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval (30 min, 6h - day 3, day 4 - 7, 6h - day 7).

13.3 Unsolicited Adverse Events

The analysis will use unsolicited adverse event data from all reporting sources combined.

All the unsolicited AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA preferred terms into frequency tables according to SOC.

All AEs will be summarized by vaccine group, according to SOC and PT within SOC. When an unsolicited AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Only vaccine-emergent AEs will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination. The selection of unsolicited adverse events and the assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

- Onset between day 1 and day 28.
- Onset between day 29 and day 57.
- Onset after day 28 through study end.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.
- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.

- Unsolicited adverse events leading to dose reduction, interruption or delay in study vaccination.
- Unsolicited adverse events leading to hospitalization.
- Unsolicited adverse events leading to new onset of chronic disease.
- Unsolicited adverse events of special interest.
- Medically attended adverse events.

Solicited adverse events continuing beyond day 7 will be coded by MedDRA and combined with the respective unsolicited adverse events.

13.4 Combined Solicited and Unsolicited Adverse Events

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA. For clintrial.gov posting purposes, a summary of combined solicited and unsolicited non-serious adverse events will be produced. A further differentiation of combined adverse events according to seriousness, severity, or relationship is not possible.

13.5 Clinical Safety Laboratory Investigations

Clinical safety laboratory values, and change-from-baseline values (study day 8, 29, 36 and 57 vs. day 1), will be summarized (mean, standard deviation, median, minimum and maximum) at each time-point of assessment, by vaccine group, for subjects in the Overall Safety Set with available laboratory data.

The frequencies of subjects with clinical laboratory values below, within, or above normal ranges will be tabulated for each clinical laboratory variable by vaccine-group and time-point of assessment (3 x 3 shift tables).

The percentages of subjects that show a ‘range change abnormal high’ (RCAH) or a ‘range change abnormal low’ (RCAL) are to be tabulated for each clinical laboratory variable by vaccine-group and time point of assessment. A RCAH is a laboratory value that is low or normal at baseline but high post-baseline. A RCAL is a laboratory value that is normal or high at baseline but low post-baseline.

Laboratory values will also be classified and tabulated according to CBER toxicity criteria (CBER 2007b, see section 19 for reference).

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges and assessment of clinical significance.

For subjects presenting at least one clinically significant value, an additional listing will be provided of all laboratory results by vaccine group, by subject, and by relevant parameter. Clinical significance assessed by the investigator will be presented.

13.6 Concomitant Medication

The frequencies and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary.

14. INTERIM ANALYSIS

14.1 Interim Analysis

An Interim Analysis to be used for internal future project planning will be performed on the immunogenicity and safety data collected from all subjects at Visits 1 (day 1), 5 (day 29), and 9 (day 57).

There will not be any statistical penalties applied as the interim analysis will not trigger changes in the study conduct (e.g. sample size re-estimation, cancellation of vaccine groups, early stop). The interim analysis will be considered final with regards to immunogenicity up to day 57.

The site staff will remain blinded through the end of the study and will not have access to unblinded information. The study statistician and the statistical programmer will be the only NVD people to be unblinded at subject level (i.e. full unblinding) for the interim analysis. Other NVD personnel will only receive grouped unblinded information with no possibility to guess the treatment allocation for a single subject.

A thorough description of how data and the blind will be secured will be reported in the Data Security Plan (DSP). An interim CSR will not be produced.

Overall safety results (i.e. tables produced only for the study as a whole and not by vaccine group) will be provided with no risk of potential unblinding. Safety listings will not be produced to avoid the risk of unblinding due to possible scarcity of events.

Data reported in the interim analysis that were later changed or modified will be provided in an appendix along with the original entries.

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

A Data Monitoring Committee (DMC) will be implemented to review safety data during scheduled periodic reviews. The DMC will review safety data, as described in the DMC charter.

With regards to timing, DMC meetings are planned:

- Soon after the first 12 subjects in each cohort receive the first vaccine dose, before proceeding with enrollment and vaccination of the remaining subjects in each cohort;
- After the two-vaccination series in each cohort has been completed and before proceeding with enrollment for the subsequent cohort.

DMC review	Decision
Cohort 1: Day 8 for 12 subjects	Complete enrollment in Cohort 1
Cohort 1: Day 57 for all subjects	Begin enrollment in Cohort 2
Cohort 2: Day 8 for 12 subjects	Complete enrollment in Cohort 2
Cohort 2: Day 57 for all subjects	Begin enrollment in Cohort 3
Cohort 3: Day 8 for 12 subjects	Complete enrollment in Cohort 3
Optional review to be determined	

16. PEER REVIEW

The type of peer review required for each output is to be identified by the study Biostatistician and SP in the TOC (see BCDM-14 TEMP 04). Analyses/Outputs requiring statistical peer review correspond usually to the analyses of the primary and secondary objectives, and data conversion programs. Peer review of these analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

The following analyses are identified as key analyses to be peer reviewed by a biostatistician independent from the study:

- Primary immunogenicity analysis.
- Primary safety analysis

The following programs are identified as key programs to be peer reviewed by a second SP:

- Exclusion file(s).
- Data conversion program.
- Primary and secondary immunogenicity analyses
- Safety analyses

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures, please refer to the Table of Contents (TOC) stored in 'Enterprise/eTMF Repository/V122_01/Cluster Documents/Statistical analysis/Statistical analysis Plan'.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TFLs are to include the following header:

Novartis Vaccines	Vaccine: RSV F Subunit (RSV101)
Final Report: Study V122_01	Two Doses in Healthy Adults

In all tables, listings and figures, vaccine groups will be labeled as follows:

Group 1A will be labeled as 'RSV F 45 No Adj'

Group 1B will be labeled as 'RSV F 45 Alum'

Group 1C will be labeled as 'RSV F 45 MF59'

Group 1D will be labeled as 'Placebo'

Group 2A will be labeled as 'RSV F 90 No Adj'

Group 2B will be labeled as 'RSV F 90 Alum'

Group 2C will be labeled as 'RSV F 90 MF59'

Group 2D will be labeled as 'Placebo'

Group 3A will be labeled as 'RSV F 135 No Adj'

Group 3B will be labeled as 'RSV F 135 Alum'

Group 3C will be labeled as 'RSV F 135 MF59'

Group 3D will be labeled as 'Placebo''

For the mock-up catalogue to be used during programming, please refer to the document stored in 'Home/analysis/V122/V122_01/final/prod/doc' within the SAS Drug Development (SDD) server.

Since all TLFs will be produced using SAS®, the output actually generated may slightly differ from the mock-ups presented in the study specific Mock-up catalogue.

19. REFERENCES

Nauta J. *Statistics in Clinical Vaccine Trials*. 2010. Heidelberg: Springer.

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*

Clopper CJ, Pearson ES. *The use of confidential or fiducial limits illustrated in the case of the binomial*. Biometrika 1934; 26:404-413.

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STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 1 Randomized, Observer Blind, Placebo Controlled, Dosage-Escalation Single Center Study to Evaluate the Safety and Immunogenicity of an RSV Fusion Glycoprotein (F) Subunit Vaccine in Healthy Adults
Study Number:	V122_01
Protocol Version and Date:	4.0, 12 APR 16
Phase of Development:	Phase 1
Sponsor:	GSK
Plan Prepared by:	PPD
Version and Date:	Version 2.0: 03 MAY 16
Approvers:	PPD, Supervisory Biostatistician
	PPD, Cluster Head
	PPD, Cluster Physician
	PPD, Clinical Trial Leader
	PPD, Medical Writer

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CSR	Clinical Study Report
CTL	Clinical Trial Leader
DMC	Data Monitoring Committee
FAS	Full Analysis Set
GSK	GlaxoSmithKline
MedDRA	Medical Dictionary for Regulatory Activities
PD	Protocol Deviation
PPS	Per Protocol Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Content

1. BACKGROUND AND RATIONALE

Respiratory syncytial virus (RSV) is the most important cause of acute lower respiratory tract infections (ALRIs) that result in hospital visits during infancy and early childhood. In the United States, more than 60% of infants are infected by RSV during their first RSV season, and nearly all have been infected by two to three years of age. Disease burden is similar in Europe; globally, among children less than five years of age, RSV causes an estimated 33.8 million ALRIs each year (more than 22% of all ALRIs), resulting in 66,000-199,000 deaths, 99% of which occur in developing countries.

In addition, RSV is a common cause of respiratory disease among the elderly, resulting in as many hospitalizations as influenza in a heavily influenza-immunized population.

GSK (GlaxoSmithKline) has developed an investigational RSV subunit vaccine from an engineered recombinant RSV fusion (F) glycoprotein. The clinical program will evaluate the feasibility of passively protecting infants by immunizing pregnant women with the RSV F subunit vaccine. If maternal immunization during 24 to 32 weeks of gestation increases antibody titers eight-fold, the median peak of RSV disease in infants would be delayed from its current peak at two to three months of age to a new peak at approximately five to six months of age. This delay could significantly decrease the burden of RSV disease in infants in the first months of life and open an RSV disease-free interval during which active immunization of infants could further extend protection beyond six months of age. In the current study, we will test one dosage of the investigational RSV F subunit vaccine that is presumed to be below the dosage-response plateau (to establish that a maximal neutralizing antibody response cannot be achieved with a low dosage) and two higher dosages (to either establish the dosage-response plateau or indicate that higher dosages are needed in a subsequent study to reach the plateau).

The purpose of this study is to evaluate the safety and immunogenicity of two doses of the investigational RSV F subunit vaccine administered intramuscularly (IM). In this current Phase 1, first-in-human study, the three different antigen amounts that have been selected will be evaluated in a stepwise manner in three different cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 µg], cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and cohort 3: high dosage of RSV F subunit vaccine [135 µg]). In addition, the effect of an adjuvant, either aluminum hydroxide or MF59, and antibody kinetics post-vaccination at different time points will be evaluated as compared to unadjuvanted RSV F subunit vaccine at the same dosage levels.

For further details please refer to section 1.0 of the protocol.

2. OBJECTIVES

2.1 Primary Objective(s)

Primary Immunogenicity Objective

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Safety Objective

1. To assess the safety of the RSV F subunit vaccine compared to placebo.

2.2 Secondary Objectives

Secondary Immunogenicity Objectives

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).
2. To evaluate the total serum antibody responses to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
3. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to the RSV protein F in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
4. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to each of the RSV proteins G and N in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

Secondary Exploratory Objectives

1. To characterize the serum neutralizing antibody (NAb) and binding antibody responses against additional RSV strains and native or engineered RSV antigens at baseline and following vaccination in a subset of subjects.
2. To determine the frequency of B cells specific for RSV proteins in a subset of subjects, and subsequently, explore the baseline immunity to RSV and the immune response to the RSV F subunit vaccine by analyzing the RSV-specific B-cell repertoire in a selected group of the subset of subjects.

3. STUDY DESIGN

This is a Phase 1, randomized, observer blind, placebo-controlled, dosage-escalation, single center study, enrolling healthy adults. In total, approximately 288 healthy non-pregnant female and male adults (18 to 45 years of age) will be enrolled in the study in the ratio of 3:1. There is a higher ratio of female to male subjects because the RSV F subunit vaccine is ultimately intended for use in pregnant women. Men are still being included in this study because the vaccine may also be tested in the future in elderly subjects.

Approximately 288 healthy subjects will be enrolled (1:1:1) in a stepwise dosage-escalation manner in to one of three cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 μg], cohort 2: middle dosage of RSV F subunit vaccine [90 μg], and cohort 3: high dosage of RSV F subunit vaccine [135 μg]). Cohort 1 will be enrolled first, followed by cohort 2, and finally cohort 3.Within each cohort subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or saline placebo, as outlined in Table 1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine, with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group and 24 placebo subjects per cohort) (Table 1).

Table 3-1: Times and Events Table

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Informed Consent	X														
Medical History	X														
Physical Exam [a]	X	X [b]		X		X [b]		X		X	X	X	X	X	
Safety Laboratory Blood draw [max: 10 mL whole blood] [c]	X	X [b]		X		X [b]		X		X					
Urinalysis [d]	X	X [b]		X		X [b]		X		X					
Pregnancy Test	X	X [b]				X [b]									
Exclusion/Inclusion Criteria	X														
Randomization		X [b]													
Serology Blood draw [max: 15 mL whole blood] [e]		X [b]				X [b]				X			X		

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
2° Exploratory Obj. Blood Draw [max: 50 mL whole blood] [f]		X [b]		X		X [b]							X		
Study Vaccine Administered [g]		V				V									
30 Minutes Post Injection Assessment (Local/ Systemic AEs, Body Temperature) [h]		X				X									
Local/Systemic AEs, Body Temperature, Other Indicators of Reactogenicity [i]		X				X									
Diary Card Training [j]		X				X									
Diary Card Dispensed [k]		X				X									
Diary Card Reminder Call [l]			X				X								

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Diary Card Reviewed and Collected [m]				X				X							
Telephone Contact for Review of Safety Data [n]					X				X						
Assess all AEs, including SAEs and NOCDs Leading or not to Study Withdrawal [o]		X		X		X		X		X	X	X	X	X	X
Assess for AESIs [p]		X		X		X		X		X	X	X	X	X	X
Prior/Concomitant Medications/vaccines [q]	X	X		X		X		X		X	X	X	X	X	
Study Termination [r]															X

a. Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log. See section 6.2 for components of physical examination by visit.
b. Procedure to be performed prior to vaccination.
c. Safety laboratory assays that will be included are listed in section 3.5.3.
d. Urinalysis testing will include at a minimum protein, glucose, and presence of red blood cells.

e. Maximal blood draw refers to volume drawn for each type of assay at each specified visit. See section 3.5.1 for greater detail regarding blood sampling volumes.
f. The first 10 subjects enrolled in each treatment group of Cohort 3 who agree to the additional blood draws will need to sign an additional consent prior to the blood draw for the secondary exploratory objective.
g. Subjects will receive two doses of vaccine or placebo according to the study randomization scheme.
h. A 30 minute post-injection local and systemic adverse event and body temperature measurement will be performed by the subject under site staff supervision at the clinic during Visit 1 and Visit 5.
i. Beginning 6 hours following study vaccine administration at Visit 1 and Visit 5, and daily thereafter through 7 days after each vaccination, solicited local and systemic adverse events including other reactions (i.e. body temperature measurements and use of analgesics/antipyretics) will be reported daily by the subject on a diary card.
j. Subjects will receive training on the diary card at Visit 1 and Visit 5.
k. The diary card will be dispensed at Visit 1 and Visit 5, and subjects will be reminded that diary cards must be returned at the next clinic visit (Visit 2 or Visit 6).
l. Site staff will contact subjects by phone 2 and 4 days after each vaccination to remind them to complete their diary cards each day, and to bring the diary cards to their next clinic visit.
m. Review of safety data captured on diary cards will be completed at Visit 2 and Visit 6. Diary cards will be collected and stored with subject files.
n. Safety data will be collected for 28 days following each vaccination. At 14 and 21 days after each dose, subjects will be interviewed by site staff using a scripted interview for collection of safety data. These safety data will be transcribed on source documents by the site staff performing the interviews.
o. All medically attended AEs that lead to an unscheduled visit to a healthcare practitioner and/or a visit to the emergency department or its equivalent will be collected for 28 days following signing of the informed consent. SAEs, NOCDs and AEs leading to study or vaccine withdrawal will be collected through 1 year after receipt of the second dose. Please see section 6.6 for greater detail regarding methods for SAE and AEs leading to study or vaccine withdrawal collection.
p. Adverse events of special interest (AESIs) will also be documented in all study subjects for the duration of the study. Monitoring for AESIs will be extended through 1 year after receipt of the second dose and will be accomplished at clinic visits or by a telephone follow-up call. A tabulation of all AESIs, categorized by MedDRA preferred terms and assessed relationship to study vaccine will be submitted as an addendum to the Clinical Study Report (CSR) if not included in the CSR.
q. Collect concomitant medications and vaccination history according to the study procedures outlined in protocol section 3.2.5 and 5.4.
r. Any subject who terminates the study after receipt of vaccine (prior to Visit 9) is recommended to undergo study-related procedures required at Visit 9. For subjects who terminate after Visit 9, a telephone contact to assess for SAEs/AEs and associated concomitant medications is required.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Subjects will be enrolled in a stepwise dosage-escalation manner into one of three cohorts:

- Cohort 1: low dosage of RSV F subunit vaccine [45 µg],
- Cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and
- Cohort 3: high dosage of RSV F subunit vaccine [135 µg]).

Within each cohort, subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or placebo, as outlined in Table 4.1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group, and 24 placebo subjects in each cohort.

Table 4.1.

Cohort	Dosage	Route of Administration	Regimen: Two Doses †		
			No Adjuvant	Al(OH)3 ‡	MF59 ‡
1	45 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
2	90 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
3	135 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		

For further details please refer to section 5.1. of the protocol.

4.1.1 Definition of Vaccination Errors

The list below provides some examples of potential errors that may occur during vaccination:

- Subjects got vaccinated with a vaccine different from the one assigned at randomization

- Subjects got vaccinated with the correct vaccine but containing a lower volume
- Subjects got vaccinated with different vaccine at the second dose.

Please see Section 7 a complete guidance on how vaccination errors are handled in the statistical analysis.

4.2 Blinding and Unblinding

For details please refer to section 3.3 of the protocol.

If a subject is unblinded during the study, it is to be reported as a major protocol deviation, except for Pharmacovigilance unblinded suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately (for details see SOP BCDM-04).

The first-line analysis excludes unblinded subject(s) in immunogenicity or efficacy statistical analyses based on the per-protocol set. The unblinded subjects will be included in the full analysis set (FAS) and safety sets.

Further details on measures taken to ensure blinding can be found in the study-specific Data Security Plan.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

For details please refer to section 7.4.2.4 of the protocol.

Sample size/power considerations are included in the study protocol. Technical details including statistical assumptions and software are given in a separate sample size memo authored by the study Biostatistician. In the same document, a statistical co-reviewer verified and documented the sample size/power considerations. This document was completed prior to finalization of the protocol and stored in "Entreprise/eTMF Repository/V122_01/Cluster Documents/Statistical analysis /Statistical Analysis Plan/Other".

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

Clinical Study Report (CSR) reportable protocol deviations (PDs) are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial.

All reportable PDs will be evaluated before unblinding and classified according to ICH into the following five categories:

- Subject developed withdrawal criteria during the study but was not withdrawn
  - Underlying medical condition forbidden by the protocol or which may influence immune response.
  - Subject had contraindication for a subsequent study vaccination but was vaccinated.
  - Concomitant infection related to the vaccine which may influence immune response.
- Subject received wrong vaccine or incorrect dose
  - Study vaccine was not administered at all
  - Vaccine administration not according to protocol.
  - Randomization failure.
- Subject took an excluded concomitant medication
  - Administration of concomitant vaccine(s) forbidden in the protocol.
  - Administration of any medication forbidden by the protocol.
- Subject randomized and did not satisfy the entry criteria
  - Subject did not meet entry criteria.
- Key study procedures missed or performed out of window
  - Randomization code was broken.
  - Subject did not comply with study vaccination schedule.
  - Subject did not provide any post-vaccination safety data.
  - Subject did not comply with blood draw schedule.
  - Serological results not available post-vaccination.

- Obvious incoherence or error in data.

CSR reportable PD will lead to exclusion of the subject or part of the subject's data from at least one analysis set.

The number of subjects in any and by PD category will be summarized by vaccine and overall and individual subject listings will be provided in an appendix.

Prior to unblinding, designated GSK staff will develop a memo that describes the PDs that lead to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Cluster Physician and will be included in the trial master file (Exclusion Memo).

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of adverse events (including death) is not considered as a PD. The missing assessments that should have otherwise been collected for that subject later in the study are also not considered as a PD.

Comments:

- Missed safety calls are not considered reportable protocol deviations.
- Implausible measurements do not lead to PD.

6.2 Determination of Protocol Deviations

Prior to unblinding, a set of listings will be provided to the Cluster Physician and the Clinical Trial Leader (CTL) for review according to SOP MON-11.

The listings will be programmed following the list presented in table in section 7.3.8, and specifically using the PD codes specified in the first column.

After the review, the Cluster Physician and the CTL will provide the Biostatistician with:

- An assessment of CSR reportable PD based on blinded clinical data review.
- An assessment of subjects without PD (e.g., premature withdrawals due to adverse event, withdrawal of consent) who should be excluded from an analysis set.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible. Therefore these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	Values ≥ 900 mm Measurements < 0 mm
Induration	Values ≥ 500 mm Measurements < 0 mm
Swelling	Values ≥ 500 mm Measurements < 0 mm

7. ANALYSIS SETS

7.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the trial, and receive a subject ID.

Demography and baseline characteristics tables as well as subject listings will be produced on the All Enrolled Set.

7.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3 Full Analysis Set (FAS) Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (FAS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (FAS 1-9).

In case of vaccination error, subjects in the FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

If a subject is unblinded during the study, he/she will be included in the FAS.

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points).
- Have no major protocol deviations leading to exclusion (see section 6.2) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see section 6.2)

In detail:

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (PPS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (PPS 1-9).

For secondary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 29 (PPS 5).
- Provide immunogenicity data for anti-RSV NAb at day 181 (PPS 12).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 29 (PPS 1-5).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 181 (PPS 1-12).
- Provide immunogenicity data for RSV proteins F, G and N at baseline (PPS 1)
- Provide immunogenicity data for RSV proteins F, G and N at day 29 (PPS 5)
- Provide immunogenicity data for RSV proteins F, G and N at day 57 (PPS 9)
- Provide immunogenicity data for RSV proteins F, G and N at day 181 (PPS 12)

In case of vaccination error, the subject is excluded from the PPS. If a subject receives a vaccine, labelled for another subject but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

A minimum of 4-fold increase in antibody titers against RSV N or G from baseline to post vaccination will be considered indicative of possible natural RSV infection. This will be considered a CSR-reportable protocol deviation leading to exclusion from the PPS with associated code 250 “Concomitant infection which may influence vaccine-specific immune responses”.

If a subject is unblinded during the study, except for SUSAR, he/she will be excluded from all PPS analyses.

7.5 Safety Set

Solicited Safety Set

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

For the by vaccination tabulations, solicited safety sets will be defined as follows:

- Solicited safety set #1 for post-injection reactions after the first dose (SOL 1)
- Solicited safety set #2 for post-injection reactions after the second dose (SOL 2)

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited adverse event data.

In this respect, a confirmation of no AE is considered as adverse event data; hence subject is to be included.

Three Unsolicited Safety Sets will be defined:

- Unsolicited Safety Set Day 1 – Day 57 (UNSOL 1-57)
- Unsolicited Safety Set Day 58 – Day 394 (UNSOL 58-394)
- Unsolicited Safety Set Day 1 – Day 394 (UNSOL 1-394)

Overall Safety Set

All subjects who are in the solicited safety set and/or in the unsolicited safety set.

Subjects providing only 30 minutes post-vaccination safety data will be reported separately in a 30 minute post-vaccination safety analysis and excluded from all other safety analysis.

In case of vaccination error, subjects will be analyzed as “treated” (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.5.1 Restricted Safety Set

Not applicable

7.6 Other Analysis Set

Not applicable

7.7 Overview of Analysis Sets by PD Code

Table 7.7-1: Safety Sets

PD code	PD Description	Study Objective/ Period	All Exposed Set	Overall Safety Set	Safety Set, Unsolicited AEs	Safety Set, Solicited AEs, Period (i), T6H-D7	Safety Set, Solicited AEs, Period (i), T6H-D3	Safety Set, Solicited AEs, Period (i), D4-D7	Safety Set, Solicited AEs, Period (i), T30m
	Exclusion code		**EXPFL**	**SAFFL**	**SSU10FL**	**SSS10FL**	**SSS11FL**	**SSS12FL**	
100	**Study vaccine not administered AT ALL**	All Study	EXC	EXC	EXC	EXC	EXC	EXC	EXC
115	**Subject did not provide any post-vaccination unsolicited safety data**	All Study			EXC				
116	**Subject did not provide any post-vaccination solicited safety data**	Period (i)				EXC	EXC	EXC	EXC

EXC = excluded from this analysis set.

Table 7.7-2: Immunogenicity Sets

PD code	PD Description	Study Objective / Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x
	Exclusion code		FAS1FL	FASxFL	FAS1xFL	PPS1FL	PPSxFL	PPS1xFL
100	Study vaccine not administered AT ALL	All Study	EXC	EXC	EXC	EXC	EXC	EXC
110	Serological results are not available	Visit 1 (baseline)	EXC		EXC	EXC		EXC
110	Serological results are not available	Visit x		EXC	EXC		EXC	EXC
112	Obvious deviation from Laboratory Manual or error in laboratory data	All Study				EXC	EXC	EXC
120	Randomization failure	All Study				EXC	EXC	EXC
120.2	Subject received another vaccine than allocated (Actual Arm different from Planned Arm)	All Study				EXC	EXC	EXC
130	Randomization code was broken	All Study				EXC	EXC	EXC
140	Vaccination not according to protocol					EXC	EXC	EXC
140.4	Incomplete vaccination series (only 1st dose administered)	All Study					EXC*	EXC
150	Administration of forbidden vaccine							
200	Subject did not meet entry criteria	All Study				EXC	EXC	EXC
220	Subject had contraindication for a subsequent study vaccination but was vaccinated	Visit 5				EXC	EXC	EXC
230	Administration of forbidden medication					EXC	EXC	EXC
240	Underlying medical condition forbidden by the protocol					EXC	EXC	EXC
250	Concomitant infection related to the vaccine which may influence immune response					EXC	EXC	EXC
260	Did not comply with study vaccination schedule					EXC	EXC	EXC

PD code	PD Description	Study Objective / Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x
	Exclusion code		**FAS1FL**	**FASxFL**	**FAS1xFL**	**PPS1FL**	**PPSxFL**	**PPS1xFL**
270	**Did not comply with blood draw schedule**					EXC	EXC	EXC
280	**Subject who developed withdrawal criteria but was not withdrawn**	Visit x					EXC	EXC
290	**Early withdrawal**	Visit x		EXC	EXC		EXC	EXC

*except from PPS 5 as deviation would occur after blood draw for day 29 assessment.
FAS = Full Analysis Set; PPS=Per Protocol Set; EXC = excluded from this analysis set.
FAS 1 and PPS 1 refer to any immunogenicity analyses including values for baseline.
FAS x and PPS x refer to any immunogenicity analyses including values for visit x (i.e. visits after V1 with immunogenicity evaluation).
FAS 1x and PPS 1x refer to any immunogenicity analyses including values for baseline and visit x.
Code 999 is not a protocol deviation but it will lead to exclusion.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The log-transformed antibody titers at the time points where a blood draw for immunogenicity is collected will be analyzed using an Analysis of Covariance (ANCOVA) model which includes the vaccine-group effect (see section 11.2) and an effect defined by the log-transformed pre-vaccination antibody titer as an independent variable. Summary tables will show adjusted geometric mean titers (GMTs) for each vaccine group.

Binary data tables will show unadjusted percentages.

8.2 Handling of Dropouts, Missing Data

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: “day 1 – day 28, day 29 – day 57, day 58 – day 394”.

For solicited adverse events, the solicited study period “30 min - day 7” will be divided into: “30 min, 6h - day 3, day 4 - day 7, and 6h - day 7”.

No imputation methods will be used to address missing values.

8.2.2 Immunogenicity Data

Missing immunogenicity values are considered MCAR and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used.

Population of analyses:

PPS will be considered the principal analysis set. The primary immunogenicity analyses will be conducted on the PPS and will be repeated using the FAS as a measure of robustness. All other immunogenicity analyses will be based on the PPS.

8.2.3 Efficacy Data

Not applicable.

8.3 Multicenter Studies

Not applicable as this is a single center study.

8.4 Multiple Comparisons and Multiplicity

Statistical tests will only be used for descriptive purposes therefore no multiplicity adjustment will be done.

8.5 Immunogenicity/Safety/Other Subsets

Not applicable.

8.6 Subgroups

Not applicable.

8.7 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

(Date of Visit 1 – Date of Birth + 1) / 365.25

Body Mass Index (kg/m^2) will be calculated using the following formula:

Weight (kg) / Height^2 (m^2)

Immunogenicity

Values below the limit of quantification (LQ = 1:100, recorded as "< LQ") will be set to half that limit (i.e., LQ/2 = 1:50).

Geometric Mean Titer

The GMT will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(t_i)}{n}\right\}}$$

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers/concentrations.

Duration in the Study

Duration in the study is defined in days as:

Last visit date (visit x)[a] – Enrollment date (visit 1) + 1

[a]or premature discontinuation date (in case of withdrawal from the study)

The duration is missing if one of the dates is missing or incomplete.

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

All adverse events will be characterized according to the date of occurrence related to the vaccination phase as follows:

- **Emergence before vaccination phase**: start date before the first date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the first date of injection of study vaccine or, adverse event increase in severity including to "serious" adverse event.

If start date is equal to the first date of injection then "timing" variable ("On injection day, before injection"/"On injection day, after injection") will be used to define whether the adverse event occur before or after the injection.

If there are several vaccinations, the adverse event will be associated with the most recent vaccination.

If an adverse event start date is missing or unknown, the adverse event will be considered as emergent.

When start and/or end dates of an adverse event are only partially known, adverse events will be categorized as emergent before, during, or after vaccination phase using the following rules:

- If the partial end date is before (<) the first study vaccination (i.e., year or year & month is/are before the first study vaccination year or year & month) then the adverse event is emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the adverse event is emergent during vaccination phase.

The **maximum event severity** is the greatest severity associated with a preferred term (PT) for a reported adverse event according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe (except for the definition of emergence).

Multiple AEs with the same PT for the same subject are counted only once.

Vaccination-related Adverse Events are those for which the relationship has been evaluated by the investigator, and recorded either as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

All laboratory measurements summarized in the CSR are those defined in the study protocol. All laboratory tests including retests and reasons for retests will be presented in the data listings for each subject. In case of multiple measurements of a laboratory parameter for a subject:

1. Before the study vaccination, only the latest measurement, with closest date before (<) the first study vaccination date, will be retained for analysis.
2. After the study vaccination, only the first measurement, with soonest date after (>) the last study vaccination date, will be retained for analysis.

Reference ranges used to categorize the results as "low" (values below the lower limit of the reference range), "normal" (values within the reference range) or "high" (values above the upper value of the reference range) will be those provided by Quintiles Laboratories, which performed the tests and provided the laboratory reports.

Prestudy, Concomitant and Post-Vaccination Medications

A **previous medication** is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date).

All other medications are **concomitant**.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule.

If the first study vaccination date is missing then the medication is considered as concomitant with the study vaccination schedule, provided that the study vaccine was administered to the subject.

For further details please refer to the technical and program specifications document stored in 'Home/analysis/V122/V122_01/final/prod/docs' within the SAS Drug Development (SDD) server.

8.8 Analysis Software

All analyses will be performed using SAS Software version 9.1 or higher.

8.9 Data Transformation

Distributions of antibodies are generally skewed to the right (Nauta, 2010). Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers will be log10-transformed. GMTs and their 95% CIs are computed by exponentiating (base 10) the least squares means and 95% CIs of the log10 titers.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All subjects who provide informed consent will be accounted for in this study. The frequencies and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will also be presented.

The time the subjects are under observation will be summarized by vaccine and overall using summary statistics (mean, SD, minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median and range, and will be calculated by vaccine group and overall.

The frequencies and percentages of subjects by sex, ethnic origin, race, entry criteria fulfilled will be presented by vaccine group and overall. Demographic data will be tabulated for the All Enrolled, FAS, PPS and Safety sets.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA S system organ class (SOC) and preferred term (PT), by vaccine group and overall. Medical history data will be tabulated for the All Enrolled Set.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled set.

11.2 Primary Objectives Analysis

Primary Objective:

To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Immunogenicity Endpoints:

- Geometric mean titer (GMT) of the serum anti-RSV neutralizing antibody(NAb) titer at Day 57 (28 days after the second dose).
- Proportion of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from Day 1 (baseline) to Day 57 (28 days after the second dose).

It is assumed that log-transformed antibody titers are normally distributed; therefore values will be logarithmically transformed (base10) and all statistical analyses on antibody levels will be performed on the logarithmic scale.

Statistical models:

GMTs:

For each treatment group, GMTs of the serum anti-RSV NAb along with their associated 95% confidence intervals (CIs) will be computed by exponentiation of the corresponding log-transformed means and 95% CIs. In addition, median, minimum and maximum values will be calculated for each vaccine group at each time-point.

Adjusted GMTs will come from an ANCOVA model including fixed factor for group and covariate adjustment for baseline value.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;
BY antigen visitnum;
class group;
model logtiter_post = group logtiter_pre;
LSMEANS group / stderr cl   OUT=LSMEANS;
```

```
RUN;
```

The factorial design of the study allows fitting a statistical model able to evaluate the impact of each factor in explaining the antibody response; the following analysis of covariance (ANCOVA) model will be utilized:

$$Y_{ijk} = \mu + a_i + \beta_j + \gamma X_{ijk} + E_{ijk}$$

where Y_{ijk} represents the log-transformed (base 10) titer of the k^{th} subject in the dosage group i (i= 45µg, 90 µg or 135 µg) and adjuvant group j (j= no adjuvant, aluminum or MF59); µ is the overall mean; a_i is the main effect of dosage i; β_j is the main effect of adjuvant j, γ is a parameter included to adjust for continuous covariate X_{ijk} representing baseline titer; E_{ijk} are error terms normally distributed with mean zero and variance C^2.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;

BY antigen visitnum;

class dosage adjuvant;

model logtiter_post = dosage adjuvant logtiter_pre;

LSMEANS dosage adjuvant / stderr tdiff cl pdiff    OUT=LSMEANS;

ESTIMATE "dose 45 vs dose 90"  dose   1 -1  0;

ESTIMATE "dose 90 vs dose 135" dose   0  1 -1;

ESTIMATE "dose 45 vs dose 135"  dose   1  0 -1;

ESTIMATE "no adj vs alum" adjuvant 1 -1 0;

ESTIMATE "alum vs MF59" adjuvant 0 1 -1;

ESTIMATE "no adj vs MF59" adjuvant 1 0 -1;

RUN;
```

where `logtiter_post` represents the log-transformed antibody value of the immunogenicity variable at a given post baseline timepoint, `dosage` indicates the amount of vaccine content, `adjuvant` the type of adjuvant (i.e none, aluminium hydroxide or

MF59) , `logtiter_pre` the log-transformed antibody baseline value of the immunogenicity variable.

Placebo will not be considered in this analysis.

Analysis of interaction:

To test for a possible effect modifier, a dosage by adjuvant interaction will be added to the previous ANCOVA model. With an overall p-value smaller than 0.05, further investigations will be conducted to better understand the degree of the interaction. Additional examinations will include point estimates and 95% CIs for the different levels (e.g. low dosage by no adjuvant, low dosage by alum and so forth) of the factors under study.

4-fold increase:

Proportions of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from baseline will be presented, for each treatment group within each cohort, together with their two-sided 95% Clopper-Pearson CIs. Group differences will not be produced.

11.3 Secondary Objectives Analysis

The same statistical model as the one used for the primary objectives will be applied. No test for interaction will be conducted for the GMT analyses.

Reverse Cumulative Distribution Functions (RCDFs) will be produced.

11.4 Exploratory Objectives Analysis

Not Applicable as exploratory objectives will not be part of the core analysis of the present study. Data for the exploratory analyses will be released after the CSR for the study will be finalized.

A separate SAP to define the analyses for the exploratory objectives will be created at a later time.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not Applicable.

12.2 Secondary Objectives Analysis

Not Applicable.

12.3 Exploratory Objectives Analysis

Not Applicable.

13. SAFETY ANALYSIS

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure.
- Solicited local and systemic adverse events and indicators of solicited adverse events.
- Unsolicited adverse events.
- Clinical Laboratory Investigations.

13.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed diary cards, irrespective of severity. The analysis will show the number of subjects with *valid data* by solicited adverse event and time point. *Valid data* in the context of the safety completeness analysis are all data entered in the diary card (including implausible values) except "Not done/unknown".

Four summaries will be produced:

1. The frequencies of subjects who provide diary cards by vaccine group and collection method (i.e., clinical visit, postal mail).
2. For each solicited adverse event, the frequencies of subjects with *valid data* will be presented by vaccine group and time point: 30 min, 6h, days 2, 3, 4, 5, 6 and 7
3. For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7
4. For each solicited adverse event, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

All analyses will be based on the 'as treated' analysis set.

13.2 Solicited Local and Systemic Adverse Events

For details please refer to section 7.1.1 of the protocol.

Only solicited local and systemic adverse events reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix.

Solicited adverse events will be reported at 30 minutes, at approximately 6 hours post-vaccination on day 1 and then daily until day 7 using structured diaries. The analyses of solicited adverse events will be done separately for 30 minutes and based on three intervals: 6h - day 3, day 4 - 7 and 6h - day 7, each without 30 minutes data. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AEs.

For erythema, induration and swelling recorded originally as diameters (mm), the following categorization will be used to summarize the data:

Grade 0 (< 25 mm), any (25-50 mm, 51-100 mm, >100 mm)

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 3 schemes described below:

- by 0.5 °C increments: <36.0, ≥36.0-<36.5, ≥36.5-<37.0, ≥37.0-<37.5, ≥37.5-<38.0, ≥38.0-<38.5, ≥38.5-<39.0, ≥39.0-<39.5, ≥39.5-<40.0, ≥40.0°C
- by 1.0 °C increments: <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C
- <38.0, ≥38.0 °C

Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as an indicator of a systemic adverse event.

The analyses will encompass summaries of the data on five levels:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).
3. Solicited adverse events, maximum event severity by event and interval (6h - day 3, day 4 -7, and 6h - day 7, each without 30 min).
4. Duration of solicited adverse events.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval (6h-Day 3, Day 4-7 and 6h-Day 7, each without 30 min).

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

For each of the time points only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse event in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator in order to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 2: Time of first onset of solicited adverse events

The **time of first onset** is defined, for each subject, for each solicited adverse event, as the time point at which the respective solicited adverse event first occurred. For erythema, induration and swelling the following threshold will be used ≥ 25 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited adverse events by vaccine group, by vaccination number, and by each time point.

The tables below show examples of a listing considering four subjects who receive two vaccinations, with post-vaccination data for a given solicited adverse event and a summary table by vaccination group.

Table 13.2-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	6 Hours	Day 2	Day 3	Day 4	…	Day 7
1	PPD	None	Severe	Moderate	None	…	None
	PPD	Mild	None	None	Moderate	…	Missing
	PPD	Moderate	Mild	None	Severe	…	Mild
	PPD	Mild	Mild	None	None	…	None
2	PPD	None	None	None	None	…	Not done
	PPD	None	Mild	Mild	Missing	…	Missing
	PPD	Severe	None	Mild	Missing	…	None
	PPD	Missing	Missing	Missing	Severe	…	Mild

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'.

Table 13.2-2: Time to First Onset of Solicited Adverse Events

Vaccine group A

Vaccination	Adverse event		Number (%) of Subjects					
			6 HRS (N=4)	DAY 2 (N=4)	DAY 3 (N=4)	DAY 4 (N=4)	…	DAY 7 (N=4)
1	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	1 (25.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	0 (0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
2	XY	n	3	3	3	2	…	2
		ANY	1 (33.3%)	1 (33.3%)	0 (0%)	1 (50.0%)	…	0 (0%)
		Mild	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (33.3%)	0 (0%)	0 (0%)	1 (50.0%)	…	0 (0%)
ANY	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (25.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)

N: no. of subjects with data at a time point across all vaccinations.
n: no. of subjects with data at a time point for that specific vaccination.

Level 3: Solicited adverse events, maximum event severity by event and interval

The **maximum event severity** will be defined if there is at least one plausible non-missing observation (excluding "Not done/unknown" and implausible values) within this time interval. Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each adverse event, followed by a summary across subjects for each vaccine. Subjects without any solicited adverse events in the interval, i.e., missing values at each of the requested time points,

will be removed from the denominator. Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 4: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema, induration and swelling. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

The following example is used to illustrate how the duration is calculated:

Suppose six subjects, who received a vaccination have the post-vaccination solicited adverse event data shown in the table below. In addition, there are unsolicited adverse event reports indicating that the adverse event in subject PPD and PPD continued until day 12 and day 8, respectively. For subject PPD the number of days is calculated as 6+5 and for subject PPD as 3+1. Missing values ('Missing') are not taken into consideration

Table 13.2-3: Example for Number of Days With Solicited Adverse Events

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
PPD	None	Severe	Moderate	None	None	None	None	2
PPD	Mild	None	None	Moderate	Moderate	Moderate	Missing	4
PPD	Moderate	Mild	None	Severe	Severe	Severe	Mild[a]	11
PPD	None	None	None	None	None	None	Not done	0
PPD	None	Mild	Mild	Missing	Missing	Missing	Missing	2
PPD	Severe	None	Mild	None	None	None	Severe[b]	4

[a] continued until day 12; [b] continued until day 8

The frequency distribution of the number of days will be provided in a summary table by vaccine group, vaccination number, and by solicited adverse event.

Level 5: Solicited adverse events, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited adverse event** is defined as "any" for a subject if he/she reports greater than "none" ≥ 25 mm, for erythema, swelling, and induration) for the respective event and "none" otherwise. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic,

any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval (30 min, 6h - day 3, day 4 - 7, 6h - day 7).

13.3 Unsolicited Adverse Events

The analysis will use unsolicited adverse event data from all reporting sources combined.

All the unsolicited AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA preferred terms into frequency tables according to SOC.

All AEs will be summarized by vaccine group, according to SOC and PT within SOC. When an unsolicited AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Only vaccine-emergent AEs will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination. The selection of unsolicited adverse events and the assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

- Onset between day 1 and day 28.
- Onset between day 29 and day 57.
- Onset after day 28 through study end.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.
- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.

- Unsolicited adverse events leading to dose reduction, interruption or delay in study vaccination.
- Unsolicited adverse events leading to hospitalization.
- Unsolicited adverse events leading to new onset of chronic disease.
- Unsolicited adverse events of special interest.
- Medically attended adverse events.

Solicited adverse events continuing beyond day 7 will be coded by MedDRA and combined with the respective unsolicited adverse events.

13.4 Combined Solicited and Unsolicited Adverse Events

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA. For clintrial.gov posting purposes, a summary of combined solicited and unsolicited non-serious adverse events will be produced. A further differentiation of combined adverse events according to seriousness, severity, or relationship is not possible.

13.5 Clinical Safety Laboratory Investigations

Clinical safety laboratory values, and change-from-baseline values (study day 8, 29, 36 and 57 vs. day 1), will be summarized (mean, standard deviation, median, minimum and maximum) at each time-point of assessment, by vaccine group, for subjects in the Overall Safety Set with available laboratory data.

The frequencies of subjects with clinical laboratory values below, within, or above normal ranges will be tabulated for each clinical laboratory variable by vaccine-group and time-point of assessment (3 x 3 shift tables).

The percentages of subjects that show a ‘range change abnormal high’ (RCAH) or a ‘range change abnormal low’ (RCAL) are to be tabulated for each clinical laboratory variable by vaccine-group and time point of assessment. A RCAH is a laboratory value that is low or normal at baseline but high post-baseline. A RCAL is a laboratory value that is normal or high at baseline but low post-baseline.

Laboratory values will also be classified and tabulated according to CBER toxicity criteria (CBER 2007b, see section 19 for reference).

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges and assessment of clinical significance.

For subjects presenting at least one clinically significant value, an additional listing will be provided of all laboratory results by vaccine group, by subject, and by relevant parameter. Clinical significance assessed by the investigator will be presented.

13.6 Concomitant Medication

The frequencies and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary.

14. INTERIM ANALYSIS

14.1 Interim Analysis

An Interim Analysis will not be performed

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

A Data Monitoring Committee (DMC) will be implemented to review safety data during scheduled periodic reviews. The DMC will review safety data, as described in the DMC charter.

With regards to timing, DMC meetings are planned:

- Soon after the first 12 subjects in each cohort receive the first vaccine dose, before proceeding with enrollment and vaccination of the remaining subjects in each cohort;
- After the two-vaccination series in each cohort has been completed and before proceeding with enrollment for the subsequent cohort.

DMC review	Decision
Cohort 1: Day 8 for 12 subjects	Complete enrollment in Cohort 1
Cohort 1: Day 57 for all subjects	Begin enrollment in Cohort 2
Cohort 2: Day 8 for 12 subjects	Complete enrollment in Cohort 2
Cohort 2: Day 57 for all subjects	Begin enrollment in Cohort 3
Cohort 3: Day 8 for 12 subjects	Complete enrollment in Cohort 3
Optional review to be determined	

16. PEER REVIEW

The type of peer review required for each output is to be identified by the study Biostatistician and SP in the TOC (see BCDM-14 TEMP 04). Analyses/Outputs requiring statistical peer review correspond usually to the analyses of the primary and secondary objectives, and data conversion programs. Peer review of these analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

The following analyses are identified as key analyses to be peer reviewed by a biostatistician independent from the study:

- Primary immunogenicity analysis.
- Primary safety analysis

The following programs are identified as key programs to be peer reviewed by a second SP:

- Exclusion file(s).
- Data conversion program.
- Primary and secondary immunogenicity analyses
- Safety analyses

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures, please refer to the Table of Contents (TOC) stored in 'Enterprise/eTMF Repository/V122_01/Cluster Documents/Statistical analysis/Statistical analysis Plan'.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TFLs are to include the following header:

GSK	Vaccine: RSV F Subunit (RSV101)
Final Report: Study V122_01	Two Doses in Healthy Adults

In all tables, listings and figures, vaccine groups will be labeled as follows:

Group 1A will be labeled as 'RSV F 45 No Adj'

Group 1B will be labeled as 'RSV F 45 Alum'

Group 1C will be labeled as 'RSV F 45 MF59'

Group 1D will be labeled as 'Placebo'

Group 2A will be labeled as 'RSV F 90 No Adj'

Group 2B will be labeled as 'RSV F 90 Alum'

Group 2C will be labeled as 'RSV F 90 MF59'

Group 2D will be labeled as 'Placebo'

Group 3A will be labeled as 'RSV F 135 No Adj'

Group 3B will be labeled as 'RSV F 135 Alum'

Group 3C will be labeled as 'RSV F 135 MF59'

Group 3D will be labeled as 'Placebo''

For the mock-up catalogue to be used during programming, please refer to the document stored in 'Home/analysis/V122/V122_01/final/prod/doc' within the SAS Drug Development (SDD) server.

Since all TLFs will be produced using SAS®, the output actually generated may slightly differ from the mock-ups presented in the study specific Mock-up catalogue.

19. REFERENCES

Nauta J. *Statistics in Clinical Vaccine Trials*. 2010. Heidelberg: Springer.

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*

Clopper CJ, Pearson ES. *The use of confidential or fiducial limits illustrated in the case of the binomial*. Biometrika 1934; 26:404-413.

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STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 1 Randomized, Observer Blind, Placebo Controlled, Dosage-Escalation Single Center Study to Evaluate the Safety and Immunogenicity of an RSV Fusion Glycoprotein (F) Subunit Vaccine in Healthy Adults
Study Number:	V122_01
Protocol Version and Date:	5.0, 12 APR 16
Phase of Development:	Phase 1
Sponsor:	GSK
Plan Prepared by:	PPD
Version and Date:	Version 3.0: 18 Oct 17
Approvers:	PPD Supervisory Biostatistician
	Not Applicable, CEPL
	PPD, CRDL
	PPD, SDL
	PPD for PPD, Medical Writer

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ALRI	Acute Lower Respiratory Tract Infection
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
CRDL	Clinical Research and Development Lead
CRF	Case Report Form
CSR	Clinical Study Report
DMC	Data Monitoring Committee
EXP	Exploratory Analysis Set
FAS	Full Analysis Set
GMT	Geometric Mean Titers
GSK	GlaxoSmithKline
ICH	International Conference on Harmonisation
IM	Intramuscularly
MBC	Memory B Cell
MedDRA	Medical Dictionary for Regulatory Activities
NAb	Neutralizing Antibody
PBMC	Peripheral Blood Mononuclear Cell
PD	Protocol Deviation
PPS	Per Protocol Set
PT	Preferred Term
RCAH	Range Change Abnormal High
RCAL	Range Change Abnormal Low
RCDF	Reverse Cumulative Distribution Function
RSV	Respiratory Syncytial Virus

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDD	SAS Drug Development
SDL	Study Delivery Lead
SOC	System Organ Class
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Contents

1. BACKGROUND AND RATIONALE

Respiratory syncytial virus (RSV) is the most important cause of acute lower respiratory tract infections (ALRIs) that result in hospital visits during infancy and early childhood. In the United States, more than 60% of infants are infected by RSV during their first RSV season, and nearly all have been infected by two to three years of age. Disease burden is similar in Europe; globally, among children less than five years of age, RSV causes an estimated 33.8 million ALRIs each year (more than 22% of all ALRIs), resulting in 66,000-199,000 deaths, 99% of which occur in developing countries.

In addition, RSV is a common cause of respiratory disease among the elderly, resulting in as many hospitalizations as influenza in a heavily influenza-immunized population.

GSK (GlaxoSmithKline) has developed an investigational RSV subunit vaccine from an engineered recombinant RSV fusion (F) glycoprotein. The clinical program will evaluate the feasibility of passively protecting infants by immunizing pregnant women with the RSV F subunit vaccine. If maternal immunization during 24 to 32 weeks of gestation increases antibody titers eight-fold, the median peak of RSV disease in infants would be delayed from its current peak at two to three months of age to a new peak at approximately five to six months of age. This delay could significantly decrease the burden of RSV disease in infants in the first months of life and open an RSV disease-free interval during which active immunization of infants could further extend protection beyond six months of age. In the current study, we will test one dosage of the investigational RSV F subunit vaccine that is presumed to be below the dosage-response plateau (to establish that a maximal neutralizing antibody response cannot be achieved with a low dosage) and two higher dosages (to either establish the dosage-response plateau or indicate that higher dosages are needed in a subsequent study to reach the plateau).

The purpose of this study is to evaluate the safety and immunogenicity of two doses of the investigational RSV F subunit vaccine administered intramuscularly (IM). In this current Phase 1, first-in-human study, the three different antigen amounts that have been selected will be evaluated in a stepwise manner in three different cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 µg], cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and cohort 3: high dosage of RSV F subunit vaccine [135 µg]). In addition, the effect of an adjuvant, either aluminum hydroxide or MF59, and antibody kinetics post-vaccination at different time points will be evaluated as compared to unadjuvanted RSV F subunit vaccine at the same dosage levels.

For further details please refer to section 1.0 of the protocol.

2. OBJECTIVES

2.1 Primary Objective(s)

Primary Immunogenicity Objective

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Safety Objective

1. To assess the safety of the RSV F subunit vaccine compared to placebo.

2.2 Secondary Objectives

Secondary Immunogenicity Objectives

1. To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).
2. To evaluate the total serum antibody responses to the RSV F subunit vaccine or placebo at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
3. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to the RSV protein F in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
4. To compare the ratio of RSV F subunit serum neutralizing antibody (NAb) titer to RSV F subunit serum total binding antibody titers to each of the RSV proteins G and N in vaccine or placebo recipients at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

Secondary Exploratory Objectives

1. To characterize the serum neutralizing antibody (NAb) and binding antibody responses against additional RSV strains and native or engineered RSV antigens at baseline and following vaccination in a subset of subjects.
2. To determine the frequency of B cells specific for RSV proteins in a subset of subjects, and subsequently, explore the baseline immunity to RSV and the immune response to the RSV F subunit vaccine by analyzing the RSV-specific B-cell repertoire in a selected group of the subset of subjects.

3. STUDY DESIGN

This is a Phase 1, randomized, observer blind, placebo-controlled, dosage-escalation, single center study, enrolling healthy adults. In total, approximately 288 healthy non-pregnant female and male adults (18 to 45 years of age) will be enrolled in the study in the ratio of 3:1. There is a higher ratio of female to male subjects because the RSV F subunit vaccine is ultimately intended for use in pregnant women. Men are still being included in this study because the vaccine may also be tested in the future in elderly subjects.

Approximately 288 healthy subjects will be enrolled (1:1:1) in a stepwise dosage-escalation manner in to one of three cohorts (cohort 1: low dosage of RSV F subunit vaccine [45 µg], cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and cohort 3: high dosage of RSV F subunit vaccine [135 µg]). Cohort 1 will be enrolled first, followed by cohort 2, and finally cohort 3.Within each cohort subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or saline placebo, as outlined in Table 4-1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine, with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group and 24 placebo subjects per cohort) (Table 4-1).

The schedule of time and events, including whether a visit is a telephone call or a clinic visit is displayed in Table 3-1.

Table 3-1: Times and Events Table

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Informed Consent	X														
Medical History	X														
Physical Exam [a]	X	X [b]		X		X [b]		X		X	X	X	X	X	
Safety Laboratory Blood draw [max: 10 mL whole blood] [c]	X	X [b]		X		X [b]		X		X					
Urinalysis [d]	X	X [b]		X		X [b]		X		X					
Pregnancy Test	X	X [b]				X [b]									
Exclusion/Inclusion Criteria	X														
Randomization		X [b]													
Serology Blood draw [max: 15 mL whole blood] [e]		X [b]				X [b]				X			X		

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
2° Exploratory Obj. Blood Draw [max: 50 mL whole blood] [f]		X [b]		X		X [b]							X		
Study Vaccine Administered [g]		V				V									
30 Minutes Post Injection Assessment (Local/ Systemic AEs, Body Temperature) [h]		X				X									
Local/Systemic AEs, Body Temperature, Other Indicators of Reactogenicity [i]		X				X									
Diary Card Training [j]		X				X									
Diary Card Dispensed [k]		X				X									
Diary Card Reminder Call [l]			X				X								

Study Period	Screen	Vaccine Dose #1 & Safety Follow-up				Vaccine Dose #2 & Safety Follow-up					Post-vaccination Follow-up				
Type of Visit	**Clinic Visit**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Reminder Call**	**Clinic Visit**	**Phone Call**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Phone Call**
Study Day	**-28 to -3**	**1**	**3, 5**	**8**	**15, 22**	**29**	**31, 33**	**36**	**43, 50**	**57**	**85**	**113**	**181**	**209**	**271, 366, 394**
Days post 1st dose		**0**	**2, 4**	**7**	**14, 21**	**28**	**30, 32**	**35**	**42, 49**	**56**					
Days post 2nd dose						**0**	**2, 4**	**7**	**14, 21**	**28**	**56**				
Study Visit Window (min-max) in Days (d)	**n/a**	**n/a**	**-1/+1**	**-1/ +4 d**	**-1/+1**	**+14 d**	**-1/+1**	**-3/ +3 d**	**-1/+1**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-7/ +7 d**	**-14/ +14 d**	**-7/ +7 d**
Visit Number	**Screening**	**1**		**2**	**3, 4**	**5**		**6**	**7, 8**	**9**	**10**	**11**	**12**	**13**	**14, 15, 16**
Diary Card Reviewed and Collected [m]				X				X							
Telephone Contact for Review of Safety Data [n]					X				X						
Assess all AEs, including SAEs and NOCDs Leading or not to Study Withdrawal [o]		X		X		X		X		X	X	X	X	X	X
Assess for AESIs [p]		X		X		X		X		X	X	X	X	X	X
Prior/Concomitant Medications/vaccines [q]	X	X		X		X		X		X	X	X	X	X	
Study Termination [r]															X

a. Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log. See protocol section 6.2 for components of physical examination by visit.
b. Procedure to be performed prior to vaccination.
c. Safety laboratory assays that will be included are listed in protocol section 3.5.3.
d. Urinalysis testing will include at a minimum protein, glucose, and presence of red blood cells.

e. Maximal blood draw refers to volume drawn for each type of assay at each specified visit. See protocol section 3.5.1 for greater detail regarding blood sampling volumes.
f. The first 10 subjects enrolled in each treatment group of Cohort 3 who agree to the additional blood draws will need to sign an additional consent prior to the blood draw for the secondary exploratory objective.
g. Subjects will receive two doses of vaccine or placebo according to the study randomization scheme.
h. A 30 minute post-injection local and systemic adverse event and body temperature measurement will be performed by the subject under site staff supervision at the clinic during Visit 1 and Visit 5.
i. Beginning 6 hours following study vaccine administration at Visit 1 and Visit 5, and daily thereafter through 7 days after each vaccination, solicited local and systemic adverse events including other reactions (i.e. body temperature measurements and use of analgesics/antipyretics) will be reported daily by the subject on a diary card.
j. Subjects will receive training on the diary card at Visit 1 and Visit 5.
k. The diary card will be dispensed at Visit 1 and Visit 5, and subjects will be reminded that diary cards must be returned at the next clinic visit (Visit 2 or Visit 6).
l. Site staff will contact subjects by phone 2 and 4 days after each vaccination to remind them to complete their diary cards each day, and to bring the diary cards to their next clinic visit.
m. Review of safety data captured on diary cards will be completed at Visit 2 and Visit 6. Diary cards will be collected and stored with subject files.
n. Safety data will be collected for 28 days following each vaccination. At 14 and 21 days after each dose, subjects will be interviewed by site staff using a scripted interview for collection of safety data. These safety data will be transcribed on source documents by the site staff performing the interviews.
o. All medically attended AEs that lead to an unscheduled visit to a healthcare practitioner and/or a visit to the emergency department or its equivalent will be collected for 28 days following signing of the informed consent. SAEs, NOCDs and AEs leading to study or vaccine withdrawal will be collected through 1 year after receipt of the second dose. Please see protocol section 6.6 for greater detail regarding methods for SAE and AEs leading to study or vaccine withdrawal collection.
p. Adverse events of special interest (AESIs) will also be documented in all study subjects for the duration of the study. Monitoring for AESIs will be extended through 1 year after receipt of the second dose and will be accomplished at clinic visits or by a telephone follow-up call. A tabulation of all AESIs, categorized by MedDRA preferred terms and assessed relationship to study vaccine will be submitted as an addendum to the Clinical Study Report (CSR) if not included in the CSR.
q. Collect concomitant medications and vaccination history according to the study procedures outlined in protocol section 3.2.5 and 5.4.
r. Any subject who terminates the study after receipt of vaccine (prior to Visit 9) is recommended to undergo study-related procedures required at Visit 9. For subjects who terminate after Visit 9, a telephone contact to assess for SAEs/AEs and associated concomitant medications is required.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Subjects will be enrolled in a stepwise dosage-escalation manner into one of three cohorts:

- Cohort 1: low dosage of RSV F subunit vaccine [45 µg],
- Cohort 2: middle dosage of RSV F subunit vaccine [90 µg], and
- Cohort 3: high dosage of RSV F subunit vaccine [135 µg]).

Within each cohort, subjects will be randomly allocated (1:1:1:1) to receive vaccine with no adjuvant, vaccine with aluminum hydroxide, vaccine with MF59, or placebo, as outlined in Table 4.1. All subjects will receive two doses of a 0.5 mL intramuscular injection of the vaccine with adjuvant (aluminum hydroxide [1 mg] or MF59 [0.25 mL; 9.75 mg squalene and surfactants]), the vaccine without adjuvant, or saline placebo. There will be approximately 24 subjects in each active treatment group, and 24 placebo subjects in each cohort.

Table 4.1.

Cohort	Dosage	Route of Administration	Regimen: Two Doses †		
			No Adjuvant	Al(OH)3 ‡	MF59 ‡
1	45 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
2	90 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		
3	135 µg	IM (0.5 mL)	24	24	24
	Placebo (saline)	IM (0.5 mL)	24		

For further details please refer to section 5.1. of the protocol.

4.1.1 Definition of Vaccination Errors

The list below provides some examples of potential errors that may occur during vaccination:

- Subjects got vaccinated with a vaccine different from the one assigned at randomization

- Subjects got vaccinated with the correct vaccine but containing a lower volume
- Subjects got vaccinated with different vaccine at the second dose.

Please see Section 7 a complete guidance on how vaccination errors are handled in the statistical analysis.

4.2 Blinding and Unblinding

For details please refer to section 3.3 of the protocol.

If a subject is unblinded during the study, it is to be reported as a major protocol deviation, except for Pharmacovigilance unblinded suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately (for details see SOP BCDM-04).

The first-line analysis excludes unblinded subject(s) in immunogenicity or efficacy statistical analyses based on the per-protocol set. The unblinded subjects will be included in the full analysis set (FAS) and safety sets.

Further details on measures taken to ensure blinding can be found in the study-specific Data Security Plan.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

For details please refer to section 7.4.2.4 of the protocol.

Sample size/power considerations are included in the study protocol. Technical details including statistical assumptions and software are given in a separate sample size memo authored by the study Biostatistician. In the same document, a statistical co-reviewer verified and documented the sample size/power considerations. This document was completed prior to finalization of the protocol and stored in PPD

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

Clinical Study Report (CSR) reportable protocol deviations (PDs) are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial.

All reportable PDs will be evaluated before unblinding and classified according to ICH into the following five categories:

- Subject developed withdrawal criteria during the study but was not withdrawn
  - Underlying medical condition forbidden by the protocol or which may influence immune response.
  - Subject had contraindication for a subsequent study vaccination but was vaccinated.
  - Concomitant infection related to the vaccine which may influence immune response.
- Subject received wrong vaccine or incorrect dose
  - Study vaccine was not administered at all
  - Vaccine administration not according to protocol.
  - Randomization failure.
- Subject took an excluded concomitant medication
  - Administration of concomitant vaccine(s) forbidden in the protocol.
  - Administration of any medication forbidden by the protocol.
- Subject randomized and did not satisfy the entry criteria
  - Subject did not meet entry criteria.
- Key study procedures missed or performed out of window
  - Randomization code was broken.
  - Subject did not comply with study vaccination schedule.
  - Subject did not provide any post-vaccination safety data.
  - Subject did not comply with blood draw schedule.
  - Serological results not available post-vaccination.

- Obvious incoherence or error in data.

CSR reportable PD will lead to exclusion of the subject or part of the subject's data from at least one analysis set.

The number of subjects in any and by PD category will be summarized by vaccine and overall and individual subject listings will be provided in an appendix.

Prior to unblinding, designated GSK staff will develop a memo that describes the PDs that lead to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Cluster Physician and will be included in the trial master file (Exclusion Memo).

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of adverse events (including death) is not considered as a PD. The missing assessments that should have otherwise been collected for that subject later in the study are also not considered as a PD.

Comments:

- Missed safety calls are not considered reportable protocol deviations.
- Implausible measurements do not lead to PD.

6.2 Determination of Protocol Deviations

Prior to unblinding, a set of listings will be provided to the Clinical Research and Development Lead (CRDL) and the Study Delivery Lead (SDL) for review according to SOP MON-11.

The listings will be programmed following the list presented in table in section 7.8, and specifically using the PD codes specified in the first column.

After the review, the CRDL and the SDL will provide the Biostatistician with:

- An assessment of CSR reportable PD based on blinded clinical data review.
- An assessment of subjects without PD (e.g., premature withdrawals due to adverse event, withdrawal of consent) who should be excluded from an analysis set.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible.

Therefore these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	**Implausible measurements**
Body temperature	≤ 33°C or ≥ 42°C
Erythema	Values ≥ 900 mm Measurements < 0 mm
Induration	Values ≥ 500 mm Measurements < 0 mm
Swelling	Values ≥ 500 mm Measurements < 0 mm

7. ANALYSIS SETS

7.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the trial, and receive a subject ID.

Demography and baseline characteristics tables as well as subject listings will be produced on the All Enrolled Set.

7.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3 Full Analysis Set (FAS) Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (FAS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (FAS 1-9).

In case of vaccination error, subjects in the FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

If a subject is unblinded during the study, he/she will be included in the FAS.

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points).
- Have no major protocol deviations leading to exclusion (see section 6.2) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see section 6.2)

In detail:

For primary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 57 (PPS 9).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 57 (PPS 1-9).

For secondary immunogenicity endpoints:

- Provide immunogenicity data for anti-RSV NAb at day 29 (PPS 5).
- Provide immunogenicity data for anti-RSV NAb at day 181 (PPS 12).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 29 (PPS 1-5).
- Provide immunogenicity data for anti-RSV NAb at baseline and day 181 (PPS 1-12).
- Provide immunogenicity data for RSV proteins F, G and N at baseline (PPS 1)
- Provide immunogenicity data for RSV proteins F, G and N at day 29 (PPS 5)
- Provide immunogenicity data for RSV proteins F, G and N at day 57 (PPS 9)
- Provide immunogenicity data for RSV proteins F, G and N at day 181 (PPS 12)

In case of vaccination error, the subject is excluded from the PPS. If a subject receives a vaccine, labelled for another subject but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

A minimum of 4-fold increase in antibody titers against RSV N or G from baseline to post vaccination will be considered indicative of possible natural RSV infection. This will be considered a CSR-reportable protocol deviation leading to exclusion from the PPS with associated code 250 “Concomitant infection which may influence vaccine-specific immune responses”.

If a subject is unblinded during the study, except for SUSAR, he/she will be excluded from all PPS analyses.

7.5 Exploratory (EXP) Immunogenicity Set

All subjects included in Cohort 3 who signed an additional exploratory objective informed consent form who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points).
- Have no major protocol deviations leading to exclusion (see section 6.2) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see section 6.2)

7.6 Safety Set

Solicited Safety Set

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

For the by vaccination tabulations, solicited safety sets will be defined as follows:

- Solicited safety set #1 for post-injection reactions after the first dose (SOL 1)
- Solicited safety set #2 for post-injection reactions after the second dose (SOL 2)

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited adverse event (AE) data.

In this respect, a confirmation of no AE is considered as adverse event data; hence subject is to be included.

Five Unsolicited Safety Sets will be defined:

- Unsolicited Safety Set 1: from the day of dose 1 to before dose 2 at Visit 5 (UNSOL 1-28)
- Unsolicited Safety Set 2: from the day of dose 2 at Visit 5 to the day of Visit 9 (UNSOL 29-57)
- Unsolicited Safety Set 3: From the day of dose 1 to the day of Visit 9 (UNSOL 1-57)
- Unsolicited Safety Set 4: The day of Visit 9 to study completion (UNSOL 58-394)
- Unsolicited Safety Set 5: Day 1 – Day 394 (UNSOL 1-394)

Overall Safety Set

All subjects who are in the solicited safety set and/or in the unsolicited safety set.

Subjects providing only 30 minutes post-vaccination safety data will be reported separately in a 30 minute post-vaccination safety analysis and excluded from all other safety analysis.

In case of vaccination error, subjects will be analyzed as “treated” (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.6.1 Restricted Safety Set

Not applicable

7.7 Other Analysis Set

Not applicable

7.8 Overview of Analysis Sets by PD Code

Table 7.8-1: Safety Sets

PD code	PD Description	Study Objective / Period	All Exposed Set	Overall Safety Set	Safety Set, Unsolicited AEs				Safety Set, Solicited AEs, Period (i),			
					Overall	After Dose x*	Day 1-57	Follow-Up	T6H-D7	T6H-D3	D4-D7	T30m
	Exclusion code		EXPFL	SAFFL	SSU10FL	SSU1xFL	SSU13FL	SSU14FL	SSS10FL	SSS11FL	SSS12FL	
100	Study vaccine not administered AT ALL	All Study	EXC	EXC	EXC	EXC	EXC	EXC	EXC	EXC	EXC	EXC
115	Subject did not provide any post-vaccination unsolicited safety data	All Study			EXC	EXC	EXC	EXC				
115.x	Subject did not provide any post-vaccination unsolicited safety data	Period (i)			EXC	EXC	EXC	EXC				
116	Subject did not provide any post-vaccination solicited safety data	Period (i)							EXC	EXC	EXC	EXC

EXC = excluded from this analysis set.
* After Dose x and SSU1xFL refer to After Dose 1 for SSU11FL when x=1, and After Dose 2 for SSU12FL when x=2.

Table 7.8-2: Immunogenicity Sets

PD code	PD Description	Study Objective/ Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x	EXP 1	EXP x	EXP 1x
	Exclusion code		**FAS1FL**	**FASxFL**	**FAS1xFL**	**PPS1FL**	**PPSxFL**	**PPS1xFL**	**EXP1FL**	**EXPxFL**	**EXP1xFL**
100	**Study vaccine not administered AT ALL**	All Study	EXC	EXC	EXC	EXC	EXC	EXC	EXC	EXC	EXC
110.1	**Serological results are not available**	Visit 1 (baseline)	EXC		EXC	EXC		EXC	EXC		EXC
110.x	**Serological results are not available**	Visit x		EXC	EXC		EXC	EXC		EXC	EXC
112	**Obvious deviation from Laboratory Manual or error in laboratory data**	All Study				EXC	EXC	EXC	EXC	EXC	EXC
120	**Randomization failure**	All Study				EXC	EXC	EXC	EXC	EXC	EXC
120.2	**Subject received another vaccine than allocated (Actual Arm different from Planned Arm)**	All Study				EXC	EXC	EXC	EXC	EXC	EXC
130	**Randomization code was broken**	All Study				EXC	EXC	EXC	EXC	EXC	EXC
140	**Vaccination not according to protocol**					EXC	EXC	EXC	EXC	EXC	EXC
140.4	**Incomplete vaccination series (only 1st dose administered)**	All Study					EXC*	EXC		EXC*	EXC
150	**Administration of forbidden vaccine**										

PD code	PD Description	Study Objective/ Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x	EXP 1	EXP x	EXP 1x
	Exclusion code		FAS1FL	FASxFL	FAS1xFL	PPS1FL	PPSxFL	PPS1xFL	EXP1FL	EXPxFL	EXP1xFL
200	**Subject did not meet entry criteria**	All Study				EXC	EXC	EXC	EXC	EXC	EXC
220	**Subject had contraindication for a subsequent study vaccination but was vaccinated**	Visit 5				EXC	EXC	EXC	EXC	EXC	EXC
230	**Administration of forbidden medication**					EXC	EXC	EXC	EXC	EXC	EXC
240	**Underlying medical condition forbidden by the protocol**					EXC	EXC	EXC	EXC	EXC	EXC
250	**Concomitant infection related to the vaccine which may influence immune response**					EXC	EXC	EXC	EXC	EXC	EXC
260.1	**Did not comply with study vaccination schedule**	Dose 1				EXC	EXC	EXC	EXC	EXC	EXC
260.x	**Did not comply with study vaccination schedule**	Dose 2					EXC	EXC		EXC	EXC
270	**Did not comply with blood draw schedule**					EXC	EXC	EXC	EXC	EXC	EXC

PD code	PD Description	Study Objective/ Period	FAS 1	FAS x	FAS 1x	PPS 1	PPS x	PPS 1x	EXP 1	EXP x	EXP 1x
	Exclusion code		**FAS1FL**	**FASxFL**	**FAS1xFL**	**PPS1FL**	**PPSxFL**	**PPS1xFL**	**EXP1FL**	**EXPxFL**	**EXP1xFL**
280	**Subject who developed withdrawal criteria but was not withdrawn**	Visit x					EXC	EXC		EXC	EXC
290	**Early withdrawal**	Visit x		EXC	EXC		EXC	EXC		EXC	EXC

*except from PPS 5 as deviation would occur after blood draw for day 29 assessment.
FAS = Full Analysis Set; PPS=Per Protocol Set; EXP=Exploratory Immunogenicity Set, EXC = excluded from this analysis set.
FAS 1, PPS 1, and EXP 1 refer to any immunogenicity analyses including values for baseline.
FAS x, PPS x, and EXP x refer to any immunogenicity analyses including values for visit x (i.e. visits after V1 with immunogenicity evaluation).
FAS 1x, PPS 1x, and EXP 1x refer to any immunogenicity analyses including values for baseline and visit x.
Code 999 is not a protocol deviation but it will lead to exclusion.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The log-transformed antibody titers at the time points where a blood draw for immunogenicity is collected will be analyzed using an Analysis of Covariance (ANCOVA) model which includes the vaccine-group effect (see section 11.2) and an effect defined by the log-transformed pre-vaccination antibody titer as an independent variable. Summary tables will show adjusted geometric mean titers (GMTs) for each vaccine group.

Binary data tables will show unadjusted percentages.

8.2 Handling of Dropouts, Missing Data

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: “day 1 – day 28, day 29 – day 57, day 58 – day 394”.

For solicited adverse events, the solicited study period “30 min - day 7” will be divided into: “30 min, 6h - day 3, day 4 - day 7, and 6h - day 7”.

No imputation methods will be used to address missing values.

8.2.2 Immunogenicity Data

Missing immunogenicity values are considered missing completely at random (MCAR) and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used.

Population of analyses:

PPS will be considered the principal analysis set. The primary immunogenicity analyses will be conducted on the PPS and will be repeated using the FAS as a measure of robustness. All other primary and secondary immunogenicity analyses will be based on the PPS.

The exploratory immunogenicity analyses will be based on the EXP.

8.2.3 Efficacy Data

Not applicable.

8.3 Multicenter Studies

Not applicable as this is a single center study.

8.4 Multiple Comparisons and Multiplicity

Statistical tests will only be used for descriptive purposes therefore no multiplicity adjustment will be done.

8.5 Immunogenicity/Safety/Other Subsets

Not applicable.

8.6 Subgroups

Not applicable.

8.7 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

(Date of Visit 1 – Date of Birth + 1) / 365.25

Body Mass Index (kg/m^2) will be calculated using the following formula:

Weight (kg) / Height^2 (m^2)

Immunogenicity

Values below the limit of quantification (LLQ = 1:100, recorded as "< LLQ") will be set to half that limit (i.e., LLQ/2 = 1:50).

Geometric Mean Titer

The GMT will be calculated using the following formula:

$$10^{\left\{\frac{\sum \log_{10}(t_i)}{n}\right\}}$$

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers/concentrations.

Ratio greater or equal to a given threshold is defined for non-missing values as:

= 1, if the concentration relative to pre-vaccination is superior or equal to the given fold-rise threshold

= 0, otherwise

Value of 4 fold-rise will be used.

Duration in the Study

Duration in the study is defined in days as:

Last visit date (visit x)[a] – Enrollment date (visit 1) + 1

[a]or premature discontinuation date (in case of withdrawal from the study)

The duration is missing if one of the dates is missing or incomplete.

Duration in the study after vaccination 1 is defined in days as:

Date of vaccination 2 (visit 5) – Date of vaccination 1 (visit 2)

Duration in the study after vaccination 2 is defined in days as:

Date of vaccination 2 follow-up (visit 9) – Date of vaccination 2 (visit 5)

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

All adverse events will be characterized according to the date of occurrence related to the vaccination phase as follows:

- **Emergence before vaccination phase**: start date before the first date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the first date of injection of study vaccine or, adverse event increase in severity including to “serious” adverse event.

If start date is equal to the first date of injection then "timing" variable ("On injection day, before injection"/"On injection day, after injection") will be used to define whether the adverse event occur before or after the injection.

If there are several vaccinations, the adverse event will be associated with the most recent vaccination.

If an adverse event start date is missing or unknown, the adverse event will be considered as emergent.

When start and/or end dates of an adverse event are only partially known, adverse events will be categorized as emergent before, during, or after vaccination phase using the following rules:

- If the partial end date is before (<) the first study vaccination (i.e., year or year & month is/are before the first study vaccination year or year & month) then the adverse event is emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the adverse event is emergent during vaccination phase.

The **maximum event severity** is the greatest severity associated with a preferred term (PT) for a reported adverse event according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe (except for the definition of emergence).

Multiple AEs with the same PT for the same subject are counted only once.

Vaccination-related Adverse Events are those for which the relationship has been evaluated by the investigator, and recorded either as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

All laboratory measurements summarized in the CSR are those defined in the study protocol. All laboratory tests including retests and reasons for retests will be presented in the data listings for each subject. In case of multiple measurements of a laboratory parameter for a subject:

1. Before the study vaccination, only the latest measurement, with closest date before (<) the first study vaccination date, will be retained for analysis.
2. After the study vaccination, only the first measurement, with soonest date after (>) the last study vaccination date, will be retained for analysis.

Reference ranges used to categorize the results as "low" (values below the lower limit of the reference range), "normal" (values within the reference range) or "high" (values above the upper value of the reference range) will be those provided by Quintiles Laboratories, which performed the tests and provided the laboratory reports.

Pre-study, Concomitant and Post-Vaccination Medications

A **previous medication** is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date).

All other medications are **concomitant**.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule.

If the first study vaccination date is missing then the medication is considered as concomitant with the study vaccination schedule, provided that the study vaccine was administered to the subject.

For further details please refer to the technical and program specifications document stored in PPD within the SAS Drug Development (SDD) server.

8.8 Analysis Software

All analyses will be performed using SAS Software version 9.1 or higher.

8.9 Data Transformation

Distributions of antibodies are generally skewed to the right (Nauta, 2010). Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers will be log10-transformed. GMTs and their 95% confidence intervals (CIs) are computed by exponentiating (base 10) the least squares means and 95% CIs of the log10 titers.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All subjects who provide informed consent will be accounted for in this study. The frequencies and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will also be presented.

The time the subjects are under observation will be summarized by vaccine and overall using summary statistics (mean, SD, minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median and range, and will be calculated by vaccine group and overall for each cohort.

The frequencies and percentages of subjects by sex, ethnic origin, race, entry criteria fulfilled will be presented by vaccine group and overall for each cohort. Demographic data will be tabulated for the All Enrolled, FAS, PPS, EXP and Safety sets.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA system organ class (SOC) and preferred term (PT), by vaccine group and overall for each cohort. Medical history data will be tabulated for the All Enrolled Set.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group for each cohort. Data will be tabulated for the All Enrolled set.

11.2 Primary Objectives Analysis

Primary Objective:

To evaluate the serum neutralizing antibody (NAb) response to the RSV F subunit vaccine or placebo at Day 57 (28 days after the second dose).

Primary Immunogenicity Endpoints:

- Geometric mean titer (GMT) of the serum anti-RSV neutralizing antibody (NAb) titer at Day 57 (28 days after the second dose).
- Proportion of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from Day 1 (baseline) to Day 57 (28 days after the second dose).

It is assumed that log-transformed antibody titers are normally distributed; therefore values will be logarithmically transformed (base10) and all statistical analyses on antibody levels will be performed on the logarithmic scale.

Statistical models:

GMTs:

For each treatment group, GMTs of the serum anti-RSV NAb along with their associated 95% CIs will be computed by exponentiation of the corresponding log-transformed means and 95% CIs. In addition, median, minimum and maximum values will be calculated for each vaccine group at each time-point.

Adjusted GMTs for adjuvant groups within each cohort (dosage level) separately will come from an ANCOVA model including fixed factor for adjuvant and covariate adjustment for baseline value.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;
BY cohort antigen visitnum;
class adjuvant;
model logtiter_post = adjuvant logtiter_pre;
LSMEANS adjuvant / stderr cl   OUT=LSMEANS;
```

```
RUN;
```

Adjusted GMTs for dosage groups within each adjuvant separately will come from an ANCOVA model including fixed factor for dosage and covariate adjustment for baseline value.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;
BY adjuvant antigen visitnum;
class dosage;
model logtiter_post = dosage logtiter_pre;
LSMEANS dosage / stderr cl   OUT=LSMEANS;
RUN;
```

The factorial design of the study allows fitting a statistical model able to evaluate the impact of each factor in explaining the antibody response; the following analysis of covariance (ANCOVA) model will be utilized:

$$Y_{ijk} = \mu + a_i + \beta_j + \gamma X_{ijk} + t_{ijk}$$

where Y_{ijk} represents the log-transformed (base 10) titer of the k^{th} subject in the dosage group i (i= 45µg, 90 µg or 135 µg) and adjuvant group j (j= no adjuvant, aluminum or MF59); µ is the overall mean; a_i is the main effect of dosage i; β_j is the main effect of adjuvant j, γ is a parameter included to adjust for continuous covariate X_{ijk} representing baseline titer; t_{ijk} are error terms normally distributed with mean zero and variance σ^2.

The following SAS® code will be used for the ANCOVA model:

```
PROC GLM data = dataset;
BY antigen visitnum;
class dosage adjuvant;
model logtiter_post = dosage adjuvant logtiter_pre;
LSMEANS dosage adjuvant / stderr tdiff cl pdiff    OUT=LSMEANS;
ESTIMATE "dose 45 vs dose 90"  dose   1 -1  0;
ESTIMATE "dose 90 vs dose 135" dose   0  1 -1;
ESTIMATE "dose 45 vs dose 135"  dose   1  0 -1;
ESTIMATE "no adj vs alum" adjuvant 1 -1 0;
ESTIMATE "alum vs MF59" adjuvant 0 1 -1;
ESTIMATE "no adj vs MF59" adjuvant 1 0 -1;
RUN;
```

where `logtiter_post` represents the log-transformed antibody value of the immunogenicity variable at a given post baseline timepoint, `dosage` indicates the amount of vaccine content, `adjuvant` the type of adjuvant (i.e none, aluminum hydroxide or

MF59) , `logtiter_pre` the log-transformed antibody baseline value of the immunogenicity variable.

Placebo will not be considered in this analysis.

Analysis of interaction:

To test for a possible effect modifier, a dosage by adjuvant interaction will be added to the previous ANCOVA model. With an overall p-value smaller than 0.05, further investigations will be conducted to better understand the degree of the interaction. Additional examinations will include point estimates and 95% CIs for the different levels (e.g. low dosage by no adjuvant, low dosage by alum and so forth) of the factors under study.

4-fold increase:

Proportions of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from baseline will be presented, for each treatment group within each cohort, together with their two-sided 95% Clopper-Pearson CIs (Clopper, 1934). Group differences will not be produced.

11.3 Secondary Objectives Analysis

Serum anti-RSV NAb titer

Secondary immunogenicity endpoints are:

1. GMT of the serum anti-RSV NAb titer at Day 1 (baseline), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).

2. Proportion of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from Day 1 (baseline) to all other time points (Day 29 [28 days after the first dose] and Day 181 [six months after the first dose]).

3. Proportion of subjects at Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose) who achieve serum anti-RSV NAb titers greater than the 3rd quartile of the serum anti-RSV NAb titers overall distribution at Day 1 (baseline).
4. Reverse cumulative distribution of serum anti-RSV NAb titers at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

The same statistical model as the one used for the primary objectives will be applied to evaluate the serum neutralizing antibody response at Day 1, Day 29 and Day 181. No test for interaction will be conducted for the GMT analyses.

Proportions of subjects with a ≥4-fold increase in serum anti-RSV NAb titer from baseline will also be presented for each treatment group within each cohort together with their two-sided 95% Clopper-Pearson CIs. Additionally, the proportions of subjects whose serum anti-RSV NAb titer at Day 29, Day 57 and Day 181 greater than the third quartile (75-th percentile) of the serum anti-RSV NAb titer at Day 1 for all subjects combined will be presented for each treatment group within each cohort, together with their two-sided 95% CIs.

Reverse Cumulative Distribution Functions (RCDFs) will be produced.

Serum total binding antibody responses

Secondary immunogenicity endpoints are:

1. GMT of the serum total binding antibody to each of the RSV proteins F, G, and N at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).
2. Proportion of subjects with a ≥4-fold increase in serum total binding antibody titers to each of the RSV proteins F, G, and N from Day 1 (baseline) to all time points (Day 29 [28 days after the first dose], Day 57 [28 days after the second dose], and Day 181 [six months after the first dose]).
3. Proportion of subjects at Days 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose) who achieve serum total binding antibody titers to each of the RSV proteins F, G, and N greater than the 3rd quartile of serum total binding antibody titers to RSV protein F at Day 1 (baseline).
4. Reverse cumulative distribution of serum total binding antibody to each of the RSV proteins F, G, and N at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

Analyses of the serum total binding antibody responses to each of the RSV proteins, F, G, and N will use the same primary ANCOVA model as for the primary objective. Similarly, the proportions of subjects with a ≥4-fold increase in serum total binding antibody titers will be analyzed in the same manner as the anti-RSV NAb titers. Additionally, the third quartile of the serum total binding antibody to each of the RSV proteins at Day 1 for all subject combined will be determined; and, the proportions of

subjects with a post-vaccination titer greater than or equal to that value will be analyzed in the same manner as the 4-fold increase analyses.

Ratio of serum NAb titer to serum total binding antibody titers

1. Ratio of RSV F subunit serum neutralizing antibody (NAb) titer to each of the RSV F subunit serum total binding antibody titers to RSV proteins F, G, and N at Day 1 (baseline), Day 29 (28 days after the first dose), Day 57 (28 days after the second dose), and Day 181 (six months after the first dose).

Analyses of the ratio of serum NAb titer to serum total binding antibody titer to each of the RSV proteins (F, G, and N) will use the same primary ANCOVA model as for the primary objective with the serum NAb titer at Day 1 as the covariate.

11.4 Exploratory Objectives Analysis

Exploratory immunogenicity endpoints are:

1. Predominant isotype of the RSV-specific serum antibody at multiple time points.
2. RSV-specific immune response against different RSV group A and group B strains or engineered RSV antigens at multiple time points.
3. Frequency of B cells secreting RSV-specific antibodies at Day 1 (baseline), Day 8 (seven days after the first dose), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).
4. Diversity of the B-cell repertoire at Day 1 (baseline), Day 8 (seven days after the first dose), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose).

The frequency of B cells secreting RSV-specific antibodies will be determined by ELISPOT at Day 1 (baseline), Day 8 (seven days after the first dose), Day 29 (28 days after the first dose), and Day 181 (six months after the first dose) in order to evaluate the baseline specific B-cell frequency (Day 1 baseline), the peak of plasmablast responses (Day 8), the peak of B cell memory responses (Day 29), and the persistence of memory B cell responses (Day 181).

Analysis Method:

The percentage of subjects with detectable frequencies of IgG+ and IgM+ RSV-MBC and HSA-MBC will be summarized using two-sided 95% Clopper-Pearson CIs by each treatment group within each cohort at days 1, 8, 29 and 181.

The number of circulating IgG+ and IgM+ RSV-specific plasmablasts (expressed as ASC/million PBMC) at Day 8 and the frequencies of circulating IgG+ and IgM+ RSV-

MBC and HSA-MBC (expressed as percentage of antigen-specific MBC/Total IgG and antigen-specific MBC/Total IgM) at Days 1, 29 and 181 will be summarized using medians and corresponding two-sided, 95% CIs around the median by each treatment group within each cohort. Summary statistics will be completed by providing minimum, maximum, first and third quartiles as well as the mean. To test for differences between treatment groups, the Kruskal-Wallis test will be performed. Pairwise testing of the treatment groups will be performed using the Hodges-Lehmann approach which provides the median of the pairwise difference of the dose groups along with its 95% CI.

When baseline day 1 values are non-zero, the fold changes of circulating MBC from Day 1 to Day 29 and to Day 181; and, the fold change from Day 29 to 181 will be summarized using medians and corresponding two-sided, 95% CIs around the median by each treatment group within each cohort. Summary statistics will be completed by providing minimum, maximum, first and third quartiles as well as the mean.

The median number of IgG+ and IgM+ plasmablasts per million PBMC (y-axis) will be plotted against treatment group (x-axis) differentiating each dosage and adjuvant group by symbol or color. Similarly, the median frequencies of IgG+ and IgM+ RSV-specific MBC (y-axis) at Day 1, Day 29 and Day 181 will be plotted against treatment group (x-axis) differentiating each dosage and adjuvant group by symbol or color.

Other exploratory endpoints may be analyzed at a later time point and included in a separate addendum to the CSR.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not Applicable.

12.2 Secondary Objectives Analysis

Not Applicable.

12.3 Exploratory Objectives Analysis

Not Applicable.

13. SAFETY ANALYSIS

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure.
- Solicited local and systemic adverse events and indicators of solicited adverse events.
- Unsolicited adverse events.
- Clinical Laboratory Investigations.

13.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed diary cards, irrespective of severity. The analysis will show the number of subjects with *valid data* by solicited adverse event and time point. *Valid data* in the context of the safety completeness analysis are all data entered in the diary card (including implausible values) except "Not done/unknown".

Four summaries will be produced:

1. The frequencies of subjects who provide diary cards by vaccine group and collection method (i.e., clinical visit, postal mail).
2. For each solicited adverse event, the frequencies of subjects with *valid data* will be presented by vaccine group and time point: 30 min, 6h, days 2, 3, 4, 5, 6 and 7
3. For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7
4. For each solicited adverse event, the frequencies of subjects *with valid data* by vaccine group, aggregated over time points: 6h - day 7

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

All analyses will be based on the 'as treated' analysis set.

13.2 Solicited Local and Systemic Adverse Events

For details please refer to section 7.1.1 of the protocol.

Only solicited local and systemic adverse events reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix.

Solicited adverse events will be reported at 30 minutes, at approximately 6 hours post-vaccination on day 1 and then daily until day 7 using structured diaries. The analyses of solicited adverse events will be done separately for 30 minutes and based on three intervals: 6h - day 3, day 4 - 7 and 6h - day 7, each without 30 minutes data. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AEs.

For erythema, induration and swelling recorded originally as diameters (mm), the following categorization will be used to summarize the data:

Grade 0 (< 25 mm), any (25-50 mm, 51-100 mm, >100 mm)

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 3 schemes described below:

- by 0.5 °C increments: <36.0, ≥36.0-<36.5, ≥36.5-<37.0, ≥37.0-<37.5, ≥37.5-<38.0, ≥38.0-<38.5, ≥38.5-<39.0, ≥39.0-<39.5, ≥39.5-<40.0, ≥40.0°C
- by 1.0 °C increments: <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C
- <38.0, ≥38.0 °C

Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as an indicator of a systemic adverse event.

The analyses will encompass summaries of the data on five levels:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).
3. Solicited adverse events, maximum event severity by event and interval (6h - day 3, day 4 -7, and 6h - day 7, each without 30 min).
4. Duration of solicited adverse events.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval (6h-Day 3, Day 4-7 and 6h-Day 7, each without 30 min).

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

For each of the time points only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse event in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator in order to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 2: Time of first onset of solicited adverse events

The **time of first onset** is defined, for each subject, for each solicited adverse event, as the time point at which the respective solicited adverse event first occurred. For erythema, induration and swelling the following threshold will be used ≥ 25 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited adverse events by vaccine group, by vaccination number, and by each time point.

The tables below show examples of a listing considering four subjects who receive two vaccinations, with post-vaccination data for a given solicited adverse event and a summary table by vaccination group.

Table 13.2-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	6 Hours	Day 2	Day 3	Day 4	…	Day 7
1	PPD	None	Severe	Moderate	None	…	None
	PPD	Mild	None	None	Moderate	…	Missing
	PPD	Moderate	Mild	None	Severe	…	Mild
	PPD	Mild	Mild	None	None	…	None
2	PPD	None	None	None	None	…	Not done
	PPD	None	Mild	Mild	Missing	…	Missing
	PPD	Severe	None	Mild	Missing	…	None
	PPD	Missing	Missing	Missing	Severe	…	Mild

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'.

Table 13.2-2: Time to First Onset of Solicited Adverse Events

Vaccine group A

Vaccination	Adverse event		Number (%) of Subjects				…	
			6 HRS (N=4)	DAY 2 (N=4)	DAY 3 (N=4)	DAY 4 (N=4)	…	DAY 7 (N=4)
1	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	1 (25.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	0 (0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
2	XY	n	3	3	3	2	…	2
		ANY	1 (33.3%)	1 (33.3%)	0 (0%)	1 (50.0%)	…	0 (0%)
		Mild	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (33.3%)	0 (0%)	0 (0%)	1 (50.0%)	…	0 (0%)
ANY	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (25.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)

N: no. of subjects with data at a time point across all vaccinations.
n: no. of subjects with data at a time point for that specific vaccination.

Level 3: Solicited adverse events, maximum event severity by event and interval

The **maximum event severity** will be defined if there is at least one plausible non-missing observation (excluding "Not done/unknown" and implausible values) within this time interval. Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each adverse event, followed by a summary across subjects for each vaccine. Subjects without any solicited adverse events in the interval, i.e., missing values at each of the requested time points,

will be removed from the denominator. Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 4: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema, induration and swelling. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

The following example is used to illustrate how the duration is calculated:

Suppose six subjects, who received a vaccination have the post-vaccination solicited adverse event data shown in the table below. In addition, there are unsolicited adverse event reports indicating that the adverse event in subject PPD and PPD continued until day 12 and day 8, respectively. For subject PPD the number of days is calculated as 6+5 and for subject PPD as 3+1. Missing values ('Missing') are not taken into consideration

Table 13.2-3: Example for Number of Days With Solicited Adverse Events

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
PPD	None	Severe	Moderate	None	None	None	None	2
PPD	Mild	None	None	Moderate	Moderate	Moderate	Missing	4
PPD	Moderate	Mild	None	Severe	Severe	Severe	Mild[a]	11
PPD	None	None	None	None	None	None	Not done	0
PPD	None	Mild	Mild	Missing	Missing	Missing	Missing	2
PPD	Severe	None	Mild	None	None	None	Severe[b]	4

[a] continued until day 12; [b] continued until day 8

The frequency distribution of the number of days will be provided in a summary table by vaccine group, vaccination number, and by solicited adverse event.

Level 5: Solicited adverse events, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited adverse event** is defined as "any" for a subject if he/she reports greater than "none" ≥ 25 mm, for erythema, swelling, and induration) for the respective event and "none" otherwise. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic,

any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval (30 min, 6h - day 3, day 4 - 7, 6h - day 7).

13.3 Unsolicited Adverse Events

The analysis will use unsolicited adverse event data from all reporting sources combined.

All the unsolicited AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA preferred terms into frequency tables according to SOC.

All AEs will be summarized by vaccine group, according to SOC and PT within SOC. When an unsolicited AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Only vaccine-emergent AEs will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination. The selection of unsolicited adverse events and the assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

- Vaccination 1 AEs, Onset between day 1 and day 28.
- Vaccination 2 AEs, Onset between day 29 and day 57.
- After either vaccination, Onset between day 1 and day 57
- Onset after day 57 through study end.
- At any time during the study, Onset after vaccination 1 on day 1

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.

- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.
- Unsolicited adverse events leading to dose reduction, interruption or delay in study vaccination.
- Unsolicited adverse events leading to hospitalization.
- Unsolicited adverse events leading to new onset of chronic disease.
- Unsolicited adverse events of special interest.
- Medically attended adverse events.

Solicited adverse events continuing beyond day 7 will be coded by MedDRA and combined with the respective unsolicited adverse events.

13.4 Combined Solicited and Unsolicited Adverse Events

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA. For clintrial.gov posting purposes, a summary of combined solicited and unsolicited non-serious adverse events will be produced. A further differentiation of combined adverse events according to seriousness, severity, or relationship is not possible.

13.5 Clinical Safety Laboratory Investigations

Clinical safety laboratory values, and change-from-baseline values (study day 8, 29, 36 and 57 vs. day 1), will be summarized (mean, standard deviation, median, minimum and maximum) at each time-point of assessment, by vaccine group, for subjects in the Overall Safety Set with available laboratory data.

The frequencies of subjects with clinical laboratory values below, within, or above normal ranges will be tabulated for each clinical laboratory variable by vaccine-group and time-point of assessment (3 x 3 shift tables).

The percentages of subjects that show a ‘range change abnormal high’ (RCAH) or a ‘range change abnormal low’ (RCAL) are to be tabulated for each clinical laboratory variable by vaccine-group and time point of assessment. A RCAH is a laboratory value that is low or normal at baseline but high post-baseline. A RCAL is a laboratory value that is normal or high at baseline but low post-baseline.

Laboratory values will also be classified and tabulated according to CBER toxicity criteria (CBER 2007b, see section 19 for reference).

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges and assessment of clinical significance.

For subjects presenting at least one clinically significant value, an additional listing will be provided of all laboratory results by vaccine group, by subject, and by relevant parameter. Clinical significance assessed by the investigator will be presented.

13.6 Concomitant Medication

The frequencies and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary.

14. INTERIM ANALYSIS

14.1 Interim Analysis

An Interim Analysis will not be performed

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

A Data Monitoring Committee (DMC) will be implemented to review safety data during scheduled periodic reviews. The DMC will review safety data, as described in the DMC charter.

With regards to timing, DMC meetings are planned:

- Soon after the first 12 subjects in each cohort receive the first vaccine dose, before proceeding with enrollment and vaccination of the remaining subjects in each cohort;
- After the two-vaccination series in each cohort has been completed and before proceeding with enrollment for the subsequent cohort.

DMC review	Decision
Cohort 1: Day 8 for 12 subjects	Complete enrollment in Cohort 1
Cohort 1: Day 57 for all subjects	Begin enrollment in Cohort 2
Cohort 2: Day 8 for 12 subjects	Complete enrollment in Cohort 2
Cohort 2: Day 57 for all subjects	Begin enrollment in Cohort 3
Cohort 3: Day 8 for 12 subjects	Complete enrollment in Cohort 3
Optional review to be determined	

16. PEER REVIEW

The type of peer review required for each output is to be identified by the study Biostatistician and Statistical Programmer (SP) in the TOC (see BCDM-14 TEMP 04). Analyses/Outputs requiring statistical peer review correspond usually to the analyses of the primary and secondary objectives, and data conversion programs. Peer review of these analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

The following analyses are identified as key analyses to be peer reviewed by a biostatistician independent from the study:

- Primary immunogenicity analysis.
- Primary safety analysis

The following programs are identified as key programs to be peer reviewed by a second SP:

- Exclusion file(s).
- Data conversion program.
- Primary and secondary immunogenicity analyses
- Safety analyses

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, figures, and listings (TFLs), please refer to the Table of Contents (TOC) stored in 'Cabinets/C.A.R.S./Clinical R&D/RSV SUBUNIT (V122)/Studies/001 (205219) V122_01/11 Statistics/11.01 Statistics Oversight/11.01.01 Statistical Analysis Plan '.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TFLs are to include the following header:

GlaxoSmithKline Biologicals	Vaccine: RSV F Subunit (RSV101)
Final Report: Study V122_01	Two Doses in Healthy Adults

In all tables, listings and figures, vaccine groups will be labeled as follows:

Group 1A will be labeled as 'RSV F 45 No Adj'

Group 1B will be labeled as 'RSV F 45 Alum'

Group 1C will be labeled as 'RSV F 45 MF59'

Group 1D will be labeled as 'Placebo'

Group 2A will be labeled as 'RSV F 90 No Adj'

Group 2B will be labeled as 'RSV F 90 Alum'

Group 2C will be labeled as 'RSV F 90 MF59'

Group 2D will be labeled as 'Placebo'

Group 3A will be labeled as 'RSV F 135 No Adj'

Group 3B will be labeled as 'RSV F 135 Alum'

Group 3C will be labeled as 'RSV F 135 MF59'

Group 3D will be labeled as 'Placebo''

For the mock-up catalogue to be used during programming, please refer to the document stored in 'PPD', within the SAS Drug Development (SDD) server.

Since all TFLs will be produced using SAS®, the output actually generated may slightly differ from the mock-ups presented in the study specific Mock-up catalogue.

19. REFERENCES

Nauta J. *Statistics in Clinical Vaccine Trials*. 2010. Heidelberg: Springer.

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*

Clopper CJ, Pearson ES. *The use of confidential or fiducial limits illustrated in the case of the binomial*. Biometrika 1934; 26:404-413.