Janssen Vaccines & Prevention B.V.

Clinical Protocol

An Exploratory, Phase 2a, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Prophylactic Efficacy of a Single Immunization of Ad26.RSV.preF Against Respiratory Syncytial Virus Infection in a Virus Challenge Model in Healthy 18 to 50 Year-Old Adults

Protocol VAC18193RSV2002; Phase 2a
Amendment 3

VAC18193 (JNJ-64400141)

EudraCT NUMBER: 2017-003194-33

Status: Approved
Date: 24 July 2018
Prepared by: Janssen Vaccines & Prevention B.V.
EDMS number: EDMS-ERI-146009250, 8.0

GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

Protocol History VAC18193RSV2002			
Document Type and *File Name*	Issued Date	Amendment Type	Comments
Initial Clinical Trial Protocol *VAC18193RSV2002_Protocol (v1)*	02-August-2017	-	Not submitted
Revised Protocol *VAC18193RSV2002_Protocol (v2)*	22-August-2017	-	-
Protocol Amendment 1 *VAC18193RSV2002_Protocol_Amend_1*	20-October-2017	Non-substantial	For details, please refer to Section Amendment 1
Protocol Amendment 2 *VAC18193RSV2002_Protocol_Amend_2*	17-November-2017	Non-substantial	For details, please refer to Section Amendment 2
Protocol Amendment 3 *VAC18193RSV2002_Protocol_Amend_3*	This document	Substantial	For details, please refer to Section Amendment 3

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PROTOCOL AMENDMENTS

Protocol Version	Issue Date
Original Protocol	22 August 2017
Amendment 1	20 October 2017
Amendment 2	17 November 2017
Amendment 3	24 July 2018

Amendments below are listed beginning with the most recent amendment.

Amendment 3 (Issued date: 24 July 2018)

The overall reason for the amendment:
The power calculations for this study were based on the AUC results observed in the placebo group in a historical study (53718678RSV2001; a study with an RSV-specific fusion inhibitor in the virus challenge model). The RT-PCR assay, used to measure VL-AUC for the primary endpoint in 53718678RSV2001, is a different assay than the one in the current study. As the current study is an exploratory study, the statistical assumptions are monitored by an unblinded monitor. During this exercise it was revealed while both assays give similar results in the higher range of the assay, the assay of the historical study gives more granularity in the lower range compared to the assay in the current study. Therefore, the peak viral load is added as a secondary efficacy endpoint as this parameter should be less sensitive to the difference in the assays compared to the AUC.

The table below gives an overview of all affected sections

Synopsis: Objectives; Endpoints
2.1.1 Objectives
2.1.2 Endpoints
11.4 Efficacy Analyses
Attachment 2: Objectives and Endpoints Table

Amendment 2 (Issued date: 17 November 2017)

The overall reason for the amendment:
Following feedback from the study site, the amendment is made to facilitate enrollment by clarifying that the smoking restriction only applies from 30 days prior to entry into the quarantine Unit through the last study-related activity, and to allow participation of subjects with a history of eczema only in childhood. Alignment of the AE severity criteria and correction of the physical description of the Ad26.RSV.preF vaccine are also made.

The table below gives an overview of the rationale for each change and all affected sections

Rationale: To clarify that the smoking restriction only applies from 30 days prior to entry into the quarantine Unit through the last study-related activity, and to remove requirement for a urine cotinine test during screening and pre-vaccination on Day -28.

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Rationale: To clarify that subjects with only a childhood history of eczema are allowed to participate in the study.

4.2 Exclusion Criteria

Rationale: To align the protocol-specified AE severity criteria for AEs not listed in the FDA toxicity tables in

Attachment 1 with the FDA severity criteria.

12.1.3 Severity Criteria

Rationale: To amend the description of the Ad26.RSV.preF vaccine.

14.1 Physical Description of Study Vaccine

Rationale: Other minor changes and corrections made throughout the protocol.

Amendment 1 (Issued date: 20 October 2017)

The overall reason for the amendment:
This amendment is made to correct inconsistencies, errors and omissions in specifications of study procedures.

The table below gives an overview of the rationale for each change and all affected sections

Rationale: To indicate that the nasopharyngeal swab/nasal wash test will only be done once during screening.

Time and Events Schedule
9.1.3 Screening Phase

Rationale: To indicate that nasopharyngeal swabs will be used for the respiratory virus screen on entry to the quarantine Unit.

Time and Events Schedule
9.1.5.1 Entry to the Quarantine Unit

Rationale: To indicate that the RVAT assessment on discharge from the quarantine Unit will be done on nasopharyngeal swabs.

Time and Events Schedule

Rationale: To add drugs of abuse screen, cotinine, and breath alcohol test on Day –28.

Time and Events Schedule
9.1.4.1 Vaccination

Rationale: To change the volume of blood for clinical laboratory assessments on Day –28, Day –21 and at the early exit visit to 10 mL.

Time and Events Schedule

Rationale: To add blood volumes for assessment of cardiac enzymes on entry to the challenge unit and on Days 3, 7 and 11.

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Rationale: To add blood collection for biochemistry and hematology assessments on discharge from the quarantine Unit and at 28 days post-challenge.

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9.2.6.2 Clinical Laboratory Tests

Rationale: To include language indicating that acute myocarditis is a rare complication of viral infection.

1.3.4 Potential Risks
Rationale: To clarify the timing of immunogenicity blood draws for overflow subjects.
9.1.9 Overflow Subjects
Rationale: To clarify that tissue and paper bag distribution for mucus weight and tissue count will start on Day –1.
9.2.3 Mucus Weight and Tissue Count
Rationale: To add appearance, color, nitrite, urobilinogen, bilirubin and leucocytes to the urinalysis parameters.
9.2.6.2 Clinical Laboratory Tests
Rationale: Other minor changes and corrections made throughout the protocol.

SYNOPSIS

An Exploratory, Phase 2a, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Prophylactic Efficacy of a Single Immunization of Ad26.RSV.preF Against Respiratory Syncytial Virus Infection in a Virus Challenge Model in Healthy 18 to 50 Year-Old Adults

Ad26.RSV.preF (JNJ-64400141) is a replication-incompetent serotype 26 (Ad26) containing a deoxyribonucleic acid (DNA) transgene that encodes for the conformation-stabilized pre-fusion F protein (pre-F) of the respiratory syncytial virus (RSV) A2 strain.

RATIONALE

This is an exploratory human challenge study, the partial aim of which is to determine the utility of the RSV challenge model in RSV vaccine development by looking for a trend in efficacy of Ad26.RSV.preF, an RSV vaccine that has shown a high degree of efficacy in preclinical challenge models. In this randomized double-blind study, healthy adult subjects will be vaccinated with either a single dose of $1x10^{11}$ vp (viral particles) of Ad26.RSV.preF or placebo and 28 days later (window between 24 to 90 days) will be inoculated by the intranasal route with RSV-A Memphis 37b virus. The prophylactic antiviral effect and the prophylactic effect on clinical symptoms of RSV infection after challenge, as well as safety and tolerability of Ad26.RSV.preF after vaccination, will be evaluated compared with placebo. This is an exploratory study as an RSV vaccine has not previously been tested in this challenge model. If the findings in this study fail to suggest any trend toward efficacy of this highly potent vaccine, it might suggest that the RSV human challenge model is not appropriate for RSV vaccine development. This study could however provide an indication of the prophylactic efficacy of Ad26.RSV.preF to prevent and or modify experimentally-induced RSV infection in healthy adults aged 18-50 inclusive who have been pre-screened for susceptibility to experimental RSV infection by measurement of RSV neutralizing antibody.

OBJECTIVES, ENDPOINTS, AND HYPOTHESIS

Objectives

Primary Objective

The primary objective is to assess a trend for the prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly to adults aged 18-50 years in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the area under the curve (AUC) over time by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) compared to placebo.

Secondary Objectives

- To assess a trend for prophylactic efficacy of a single dose of Ad26.RSV.preF in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the peak viral load of the RT-PCR compared to placebo.
- To assess the effect of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF on viral load, as measured by RT-PCR and quantitative culture of RSV, and clinical symptoms on Day 6 and Day 7 post-challenge compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two quantifiable RT-PCR measurements and one or more positive clinical symptoms of any grade from two different categories in the symptom scoring system (Upper Respiratory, Lower Respiratory, Systemic), or one Grade 2 symptom from any category compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two

quantifiable RT-PCR measurements and one or more positive clinical symptoms of any grade in any category from the symptom scoring system compared to placebo.

- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of the weight of mucous secretions and tissue count over time compared to placebo.
- To assess the safety and tolerability of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly.

Exploratory Objectives

- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of clinical symptoms as measured by the AUC over time of symptoms collected by a graded symptom scoring system compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of AUC for viral load (VL-AUC), as measured by quantitative culture of RSV from nasal wash samples, compared to placebo.
- To explore the relationship of humoral (including nasal wash samples if feasible) and cellular immunogenicity outcomes and the VL-AUC and the AUC for clinical symptoms.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two quantifiable RT-PCR measurements and/or positive serological measurement of RSV infection in the context of immunization and pre-existing antibody and one or more positive clinical symptoms of any grade in any category compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of respiratory parameters measured by spirometry during the study compared to placebo.
- To explore the relationships between clinical symptoms and viral load, as measured by RT-PCR and quantitative culture of RSV, in Ad26.RSV.preF subjects and placebo.
- To explore other immunologic parameters in relation to the VL-AUC by RT-PCR and AUC for clinical symptoms and occurrence of symptomatic RSV infection defined as two quantifiable RT-PCR measurements or more plus one clinical symptom of any grade.
- To explore the immune response of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF compared to placebo.
- To explore humoral and cellular responses to challenge with RSV-A Memphis 37b in immunized and non-immunized subjects.
- To explore the relationship between immunogenicity and pre-existing Ad26 neutralizing antibody.
- To explore other potential efficacy endpoints, as defined in the statistical analysis plan (SAP), to determine the best efficacy endpoint to use in subsequent clinical studies investigating the vaccine's efficacy.

Endpoints

Primary Endpoint: Viral Load

The primary endpoint is the VL-AUC of RSV-A Memphis 37b as determined by quantitative RT-PCR assay of nasal wash samples.

Nasal wash samples are taken every 12 (±1) hours beginning two days (ie, in the morning) after inoculation of the challenge virus. Quantitative RT-PCR is utilized to measure viral load.

Secondary Endpoints

- Peak viral load of RSV-A Memphis 37b, defined as the maximum viral load as determined by quantitative RT-PCR assay of nasal wash samples, observed over the entire time period.
- Occurrence of symptomatic RSV infection defined as two quantifiable RT-PCR measurements at different timepoints plus symptoms of any grade from two different categories from the subject symptom card (SSC) or two quantifiable RT-PCR measurements plus any Grade 2 symptom from any category. The three SSC categories are:
  - Upper Respiratory symptoms: runny nose, stuffy nose, sneezing, sore throat, earache
  - Lower Respiratory symptoms: cough, shortness of breath, chest tightness, wheeze
  - Systemic symptoms: malaise, headache, muscle and/or joint ache, chilliness/feverishness
- Occurrence of RSV infection defined as two quantifiable RT-PCR measurements plus any clinical symptom of any severity.
- Total weight of mucus produced and tissue count.
- Safety and tolerability
  - Unsolicited adverse events (AEs) from informed consent form (ICF) signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge.
  - Safety data including, but not limited to, physical examinations, vital signs, 12-lead electrocardiograms (ECGs) and clinical laboratory results (including biochemistry, hematology, and urinalysis).
  - Serious adverse events (SAEs) throughout the study (from signing the ICF to the end of the study, 6 months after vaccination).
  - Solicited local and systemic AEs for 7 days after vaccination.

Exploratory Endpoints

- AUC of the total clinical symptom score. The total clinical symptom score is a composite of 13 self-reported symptoms (on the SSC) divided into three categories (Upper Respiratory, Lower Respiratory, Systemic).
- The VL-AUC of RSV-A Memphis 37b, as determined by quantitative culture of RSV of nasal wash samples.
- Pre-F antibody in nasal wash samples measured by pre-F enzyme-linked immunosorbent assay (ELISA) normalized to a standard protein or antibody to account for variability of sample volume, dilution and concentration in nasal wash samples.
- Proportion of subjects with symptomatic RSV infection (RT-PCR or serology). Seroconversion is defined as a four-fold rise against the G and/or N protein by ELISA which would indicate a take of the virus inoculum to such extent that a systemic immunologic response against an antigen not in the vaccine was induced.
- Neutralizing antibody against RSV-Memphis 37b (challenge strain) and RSV A2.
- RSV pre-F and post-F antibodies from serum and nasal wash samples[a] measured by ELISA.

[a] Assumes that the ELISA for nasal wash samples will be available.

- Respiratory parameters: spirometry (FEV1, FVC, FEV1/FVC).
- Vaccine immune response defined as specific pre-F levels of antibody as measured by ELISA, specific neutralizing antibody levels, F antigen-specific cellular responses as measured by interferon gamma enzyme-linked immunospot (IFNγ ELISpot) assay, flow cytometry cytokine analysis and/or secreted cytokines compared to placebo.

Additional exploratory analyses may be performed to investigate vaccine-elicited immune responses further. These may include, but are not limited to, the following assays:

- RSV cross-neutralization of B and/or other A strain
- Binding antibodies to RSV G and/or N
- F-protein antibody specificity characterization
- Adenovirus neutralization assay
- Functional and molecular antibody characterization

Hypothesis

This is an exploratory human challenge study that will evaluate the effect of Ad26.RSV.preF on viral replication and on clinical symptoms of RSV infection after challenge. To focus interpretation, a formal primary hypothesis is described that will be statistically tested. The primary hypothesis is that a single dose of $1x10^{11}$ vp of Ad26.RSV.preF shows a trend of reduction in VL-AUC of the quantitative RT-PCR in healthy subjects challenged intranasally with the RSV-A Memphis 37b virus, compared with healthy subjects given placebo who are similarly challenged.

As no RSV vaccine has been previously tested in a human challenge model, the statistical results of this hypothesis test will need to be interpreted with care as the relation between the results from this human challenge model and vaccine efficacy is unknown. An effect that is significant at 5% (one-sided) will be considered a significant effect. An effect that is significant at 20% (one-sided) will be considered as a trend. This trend, if observed, should be confirmed in future studies.

OVERVIEW OF STUDY DESIGN

This is a single center, randomized, placebo-controlled, double-blind Phase 2a human challenge study, to be conducted in approximately 44 healthy male and female subjects aged 18-50 years inclusive who have been pre-screened for susceptibility to RSV infection, ie, have levels of RSV neutralizing antibodies compatible with susceptibility to RSV infection[a].

Subjects will receive single intramuscular doses of $1x10^{11}$ vp of Ad26.RSV.preF or placebo. More than 44 (and up to 70) subjects will be vaccinated to account for withdrawals between vaccination and challenge. The challenge study site, hVIVO, has the capacity to challenge and house under isolation 22 subjects at a time and thus the study will be conducted in several cohorts with up to 22 subjects per cohort. Within each cohort, subjects will be randomized 1:1 to $1x10^{11}$ vp of Ad26.RSV.preF or placebo.

[a] The cut-off is based on the average 25th percentile of the past 12 months screening results.

Table 1: Study Design: Vaccination and Challenge

Group	N	Day –28	Day 0*
1	22	Ad26.RSV.preF ($1x10^{11}$ vp)	Challenge with RSV-A Memphis 37b**
2	22	Placebo	

* ie, not less than 24 or more than 90 days after vaccination.

**Subjects will be challenged in two or more cohorts of up to 22 subjects per cohort. Within each cohort, subjects will be randomized 1:1 to $1x10^{11}$ vp of Ad26.RSV.preF or placebo.

Note: The infection rate (based on mucus weight, symptoms and viral shedding) in the placebo group will be followed on an ongoing basis by an unblinded individual who does not have any other study function to ensure a sufficient infection rate has occurred. If the infection rate is lower than anticipated, additional subjects may be enrolled, initially four additional subjects in each arm and up to 70 in total. This is to ensure that a high enough number of evaluable subjects is reached in each arm.

Initially, two sentinel subjects will be enrolled and vaccinated, and 48-hour safety will be checked by the investigator/study-responsible physician after vaccination. Safety data for review will include solicited and unsolicited AEs and SAEs. In the absence of significant findings, the rest of the subjects will be enrolled and vaccinated. The sentinel approach with 2 subjects (1 active and 1 placebo) is taken to ensure active and placebo subjects are included in the evaluations, taking into account the blinding. The observation at 48 hours post-dose is primarily to rule out anaphylaxis or cytokine storm.

All subjects will be closely observed for a minimum of 30 minutes post-vaccination, to monitor for the development of any acute reactions, or longer if deemed necessary by the investigator. Any unsolicited, solicited local or systemic AEs and vital signs will be documented by study-site personnel following this observation period. Subjects will be given a thermometer, ruler and daily assessment (subject) diary with instructions for the proper recording of events. Each subject will record solicited local (at injection site) and systemic AEs and body temperatures, beginning on the evening of the study vaccine dosing day and on a daily basis for the following 7 days. Body temperatures should be taken at approximately the same time each day, preferably in the evening.[a] Study-site personnel will collect and review subject diary information and confirm the entries at subsequent site visits.

One to two days prior to viral challenge, subjects will be admitted to the Unit to confirm eligibility for challenge and to orient them to the challenge Unit. At this time, assessments will include relevant medical information since vaccination, physical examination, ECG and clinical laboratory testing. Blood will be collected for immunogenicity assessments. The target day for intranasal challenge with the RSV-A Memphis 37b virus is Day 0, 28 days after vaccination, with a window of 24 days to 90 days after vaccination. The 90 day window is to account for the few "overflow" subjects that were vaccinated (to be available to replace any withdrawals) but may not have been challenged but might be available for a subsequent challenge cohort as explained in more detail below.

This range for timing of the challenge is specified for the following reason. A total of 26 subjects will be vaccinated for the first challenge cohort, of whom the first 22 who are vaccinated will be challenged. Any subjects that are vaccinated but not challenged in the first cohort (overflow subjects) will be included in a subsequent cohort if they are still available and continue to meet all entry criteria. The withdrawal rate between vaccination and challenge for the first cohort will be used to assess how many extra subjects will need to be vaccinated for subsequent cohorts.[b] The durability of the humoral and cellular immune

[a] The 7-day post-vaccination temperature measurements may be taken earlier to coincide with the corresponding clinic visit.

[b] Any overflow subject who is vaccinated but not challenged will be followed to the end of the study, or can be challenged at the discretion of the sponsor, providing study blind had not been broken. Immunogenicity blood draws for overflow subjects who are not challenged will occur at 28 days post-vaccination, and again 28 days

response after a single dose of an Ad26.RSV.FA2-vectored RSV vaccine[a] was demonstrated for at least 6 months following vaccination and justifies this time window between vaccination and challenge. The 6-month immunogenicity data for Ad26.RSV.preF from study VAC18193RSV1003 will be available prior to vaccination and will be reviewed to further justify this strategy.

In the Unit, subjects will live in separate isolation rooms, and all personnel having contact with the subjects will wear isolation clothing, including personal protective equipment, to minimize the possibility of cross-contamination between subjects. On Day 0, each subject will receive the contents (0.8 mL) of 1 vial of virus inoculum.

From Day 2 to Day 11, nasal wash samples will be collected every 12 (±1) hours, abbreviated physical examination and vital signs measurements will be conducted daily, and SSCs will be filled out three times daily and reviewed by the attending physician. 24-hour tissue counts and mucus weights will be determined.

Nasopharyngeal samples will be taken on Day 12 and tested for the presence of virus prior to discharge using rapid virus antigen test (RVAT). Subjects will only be discharged on Day 12 if no detectable virus is present (negative RVAT). If symptoms are present, but no virus is detected (negative RVAT), discharge will be at the investigator's discretion. If appropriate, subjects may reside in quarantine for an additional night or longer before discharge. It is unlikely that subjects will transmit RSV virus to their close contacts after they have been released from the quarantine Unit. After infection with RSV, infectious virus will be present in the subject's nose for several days, but it is not expected to be present in the subject's nose at the time of discharge from the Unit. This is because the usual duration of time that RSV remains infective in adults is several days shorter than the time they will spend in the Unit in isolation.[9] Furthermore, at discharge, subjects will be negative by the RVAT assay.

To reduce any risk of passing RSV on to others, subjects will be advised to avoid contact with the following vulnerable groups of people for two weeks after they leave the Unit:

- any person with any known immunodeficiency
- any person receiving immunosuppressant medications
- any person undergoing or soon to undergo cancer chemotherapy
- any person who has congestive heart failure or is in frail health
- any person who has a diagnosis of emphysema or chronic obstructive pulmonary disease (COPD), is elderly residing in a nursing home, or has severe lung disease
- any person who has received a transplant (bone marrow or solid organ)
- children below the age of 1 year
- elderly persons (aged 75 years or older)

Unsolicited AEs will be collected from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. SAEs will be collected from ICF signature until 6 months after vaccination. Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

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after the date they would have originally been challenged on, to provide some vaccinated but not challenged controls for the study.

[a] See Section 1.1 for background information and clinical data on the Ad26.RSV.FA2 vaccine.

Blood will be collected for sero-susceptibility (neutralizing antibody to RSV-A Memphis 37b) within 90 days of vaccination and on entry to the Unit. Blood and urine will be collected for laboratory safety assessments within 56 days of vaccination, pre-vaccination, at 7 days post-vaccination and prior to challenge on Day –1 or Day –2.

Blood samples for humoral and cellular immunity will be collected pre-vaccination, 1-2 days prior to challenge, and at 28 days after challenge. *Note*: blood samples for immunogenicity assessment will be collected from all subjects whether subjects are challenged or not (ie, including "overflow" subjects). Blood draws for overflow subjects who are not challenged will occur at 28 days post-vaccination and again 28 days later. Overflow subjects who are challenged in a later cohort will also have blood draws at the time of challenge and 28 days after challenge.

RSV pre-F antibody will be assessed on the day before challenge and at discharge from the Unit from nasal wash samples.

The end of the study will be the last subject's last visit by telephone at 6 months post-vaccination.

An internal data review committee (DRC) will be commissioned for this study, the members of which consist of a clinician with expertise in vaccines and infectious diseases, a medical safety officer and a statistician, none of whom will be part of the study team or involved in the conduct of the study. The DRC will meet if, in the judgment of the investigator and/or the sponsor's study-responsible physician, a significant or unexpected safety event occurs.

SUBJECT POPULATION

Subjects will be healthy (on the basis of physical examination, medical history and vital signs measurement performed within 56 days of vaccination) male or female adults, aged ≥18 to ≤50 years on the day of ICF signature. All subjects will be pre-screened within 90 days of vaccination for susceptibility to RSV infection and will have RSV neutralizing antibody levels compatible with susceptibility to RSV infection.

DOSAGE AND ADMINISTRATION

Ad26.RSV.preF will be supplied at a concentration of $1x10^{11}$ vp/0.5 mL in single-use vials, with an extractable volume of 0.5 mL, for intramuscular injection into the deltoid of the non-dominant arm. A dose level of $1x10^{11}$ vp will be used.

Placebo will be supplied as sterile 0.9% saline for injection.

For each subject, every vaccination will be 0.5 mL in volume.

An unblinded pharmacist, or other qualified individual will prepare the appropriate vial and syringe and provide the syringe in a blinded manner to the study vaccine administrator who will perform the injection.

IMMUNOGENICITY EVALUATIONS

Humoral and cellular immunogenicity evaluations are summarized in the tables below. Sample collection and processing will be performed by the staff at the clinical site according to current versions of approved standard operating procedures.

Table 2: Summary of Immunogenicity Assays (Humoral)

Assay	Purpose
Exploratory endpoints	
RSV neutralization RSV-A Memphis 37b	Measure levels of the antibody to the challenge strain
Intranasal pre-F and post-F antibody*	Measure levels of vaccine induced antibody at the site of infection
RSV neutralization A2	Analysis of neutralizing antibodies to the A2 strain
RSV strain cross-neutralization	Analysis of cross-neutralizing antibodies to B and/or a different A strain
F-protein antibody (ELISA; pre- and/or post-fusion)	Analysis of antibodies binding to RSV F protein in post-fusion and/or pre-fusion form
RSV G/N ELISA	Analysis of binding antibodies to G and N proteins of RSV
Adenovirus neutralization assay	Analysis of neutralizing antibodies to adenovirus
Functional and molecular antibody characterization	Analysis of antibody characteristics including but not limited to ADCC, ADCP, avidity, Fc characteristics, Ig isotype

*From nasal wash samples, if the ELISA will be available
ADCC = antibody-dependent cell-mediated cytotoxicity; ADCP = antibody-dependent cellular phagocytosis; ELISA = enzyme-linked immunosorbent assay; F = fusion; Ig = immunoglobulin; RSV = respiratory syncytial virus

Table 3: Summary of Immunogenicity Assays (Cellular)

Assay	Purpose
Exploratory endpoints	
IFNγ ELISpot	T-cell IFNγ responses to RSV F-protein peptides
Flow cytometry (ICS)	Analysis of T-cell responses to RSV F-protein peptides (including, but not limited to, CD4/CD8, IL2, IFNγ, TNFα and/or activation markers, memory, Th1/Th2 subtyping)
Cytokine analysis	Analysis of secreted cytokines in RSV F peptide-stimulated PBMC supernatant, including, but not limited to, measurement of Th1/Th2 cytokine balance

ELISpot = enzyme-linked immunospot; F = fusion; ICS = intracellular cytokine staining; IFNγ = interferon gamma; IL = interleukin; PBMC = peripheral blood mononuclear cell; Th = T-helper (cell); RSV = respiratory syncytial virus; TNFα = tumor necrosis factor alpha

SAFETY EVALUATIONS

On a daily basis, for 7 days post-vaccination, subjects will be asked to record symptoms of the following AEs via the subject diary:

- Solicited local AEs: erythema (measured using the ruler supplied), swelling/induration (measured using the ruler supplied, and graded using the functional scale), and pain/tenderness.
- Solicited systemic AEs: fatigue, headache, myalgia, arthralgia, chills, nausea and fever (ie, body temperature ≥38 °C).

Body temperature (tympanic or oral) should be measured at approximately the same time each day using the thermometer supplied. The 7-day post-vaccination temperature measurements may be taken earlier to coincide with the corresponding clinic visit.

The investigator will review each subject's diary at the subsequent in-clinic visit; diary information will be transcribed by the study personnel into the electronic case report form (eCRF).

The investigator or study-site staff will document any reported unsolicited AEs and perform causality evaluations from the time of ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. SAEs will be collected from the time of ICF signature until the study end, 6 months after vaccination.

From the day of challenge until discharge from the Unit, a SSC will be filled out three times a day and reviewed by the attending physician. Temperature, heart rate and blood pressure will be obtained on a daily basis.

STATISTICAL METHODS

Sample Size Determination

The sample size was based on the ability to detect trends with a high one-sided alpha (20%), given this is an exploratory study whose partial aim is to determine the utility of the RSV human challenge model for RSV vaccine development. For a one-sided alpha level of 20%, the study has at least 80% power if Ad26.RSV.preF induces a reduction of VL-AUC of the quantitative RT-PCR assay by 50% or more. For a one-sided alpha level of 5%, the study has at least 80% power if Ad26.RSV.preF induces a reduction of VL-AUC of the quantitative RT-PCR assay by 75% or more. These calculations assume 22 subjects per arm, a mean VL-AUC in the placebo group of 320 $\log_{10}$ h/mL, corresponding standard deviation 275, and an infection rate in placebo of 65%.

An effect that is significant at 5% (one-sided) will be considered a significant effect. An effect that is significant at 20% (one-sided) will be considered as a trend. This trend, if observed, might be confirmed in future studies.

As this is an exploratory study, the statistical assumptions described above will need to be demonstrated to be correct for the analysis to be conducted as indicated below and as planned in further detail in the SAP. For example, if the infection rate from the study is considerably below 65% then the study will be considered to be underpowered to be able to accurately determine the stated aims.

Planned Analyses

The following analyses will be performed:

- PRIMARY ANALYSIS will be performed when all subjects have completed the quarantine phase or discontinued earlier. The pre-challenge neutralization and pre-F ELISA, and post-challenge RT-PCR, nasal mucous and tissue count, and clinical symptom data will be available although the analysis may be performed on snapshot data. All other data available at the time of primary analysis, including completed cellular data, will be included. Preliminary safety data, including Day 28 post-challenge, if available, will also be included.
- FINAL ANALYSIS will be performed when all subjects have completed the 6 month safety follow-up visit or discontinued earlier.

Depending on when the data are available, both analyses might be combined.

Efficacy Analyses

For the primary efficacy endpoint (VL-AUC of RSV-A Memphis 37b as determined by quantitative RT-PCR assay of nasal wash samples) the exact Wilcoxon Rank Sum test will be performed and the one-sided p-value will be interpreted at the 5% and 20% significance level, specifically only analyzing the statistical significance of a reduction in VL-AUC in the active versus placebo groups.

Secondary endpoints will be analyzed descriptively.

Immunogenicity Analyses

No formal hypothesis on immunogenicity will be tested. Descriptive statistics (geometric mean and 95% confidence interval [CI] for ELISA and virus neutralization assays; median and quartiles for ELISpot and intracellular cytokine staining [ICS]) will be calculated for continuous immunologic parameters at all

timepoints. For the humoral assays, geometric mean fold rises from baseline and corresponding 95% CIs may be calculated as well. For immunogenicity, baseline is considered as the last assessment pre-vaccination with Ad26.RSV.preF or placebo. Graphical representations of immunologic parameters will be made as applicable.

Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters as applicable.

Safety Analyses

No formal statistical testing of safety data is planned. For the planned analyses, safety data will be analyzed descriptively.

TIME AND EVENTS SCHEDULE

Clinic Phase	*Screening* [d]	*VACCINATION*			*VIRAL CHALLENGE: Quarantine Unit*					*OUTPATIENT*			
					Pre-viral Challenge		*Chall* [a]	*Post-viral Challenge*		*Discharge*	*Follow-up*	Final Visit [g]	
Visit Timing		**Vacc**	**Vacc +3 d** [b]	**Vacc +7 d**	**Vacc +26 d**	**Vacc +27 d**	**Vacc +28 d**	**Chall +1 d**	**Chall +2 d to +11 d**	**Chall +12 d** [c]	**Challenge + 28d**	**Vacc + 6 mo**	
Target Study Day	**Days -84 to -31** [f]	**Day -28**	**Day -25** [g]	**Day -21**	**Day -2 or Day -1** *Admission to Unit (Procedures on either day)*		**Day 0** *Viral challenge*	**Day 1**	**Day 2 onwards** *Procedures to be executed daily*	**Day 12**	**Day 28**	**Day 155**	**Early Exit** [e]
Visit Window			**±1 d**	**±2 d**							**±4 d**	**±14 d**	
Written informed consent	●	● [h]											
Inclusion/exclusion criteria	●												
Demographics	●												
Medical history/pre-study meds	●												
Medical information review					●								
Body height	●												
Body weight	●				●					●			
Complete physical examination	●				●					●			
Abbreviated/directed phys examination [i]		❶		●			●	●	●		●		●
Vital signs [j] incl. body temperature	●	❷		●	●		●	●	●	●	●		●
12-lead ECG [k]	●				●				❾				
Drugs of abuse screen	●	❶			●								
Cotinine screen					●								
Breath alcohol test	●	❶			●								
Serology (HIV-1/2, hepatitis B/C)	❸												
RSV sero-susceptibility [l]	●				●								
Nasopharyngeal swab/nasal wash tolerance test	●												
Respiratory virus screen (from nasopharyngeal swab)					●								
Serum pregnancy test [m]	●				●								●
Urine pregnancy test [m]		❶								●	●		
Randomization		❶											
Inclusion/exclusion criteria check [n]		❶											
Eligibility for viral challenge check					●								
Pre-vaccination symptoms [o]		❶											
Safety lab blood sample, mL	❸ 10	❶ 10		● 10	● 10				❿ 10	● 10	● 10		❺ 10
Urine sample for urinalysis	●	●		●	●								●
Blood sample for cellular immunity, mL		❶ 50			●50						● 50		❹ 50
Blood sample for humoral immunity, mL		❶ 10			●10						● 10		❹ 10
RSV pre-F antibody assessment (from nasal wash sample)					●					●			
Vaccination		●											
30 minute post-vaccination observation [p]		●											
Subject diary distribution [r]		●											
Subject diary review by site staff			❻	●									

Clinic Phase	*Scree-ning* [d]	*VACCINATION*			*VIRAL CHALLENGE: Quarantine Unit*						*OUTPATIENT*		
					Pre-viral Challenge		*Chall* [a]	*Post-viral Challenge*		*Dis-charge*	*Follow-up*	*Final Visit* [g]	
Visit Timing		**Vacc**	**Vacc +3 d** [b]	**Vacc +7 d**	**Vacc +26 d**	**Vacc +27 d**	**Vacc +28 d**	**Chall +1 d**	**Chall +2 d to +11 d**	**Chall +12 d** [c]	**Challenge + 28d**	**Vacc + 6 mo**	
Target Study Day	**Days -84 to -31** [f]	**Day -28**	**Day -25** [g]	**Day -21**	**Day -2 or Day -1** ***Admission to Unit (Procedures on either day)***		**Day 0** ***Viral chall-enge***	**Day 1**	**Day 2 onwards** ***Procedures to be executed daily***	**Day 12**	**Day 28**	**Day 155**	**Early Exit** [e]
Visit Window			**±1 d**	**±2 d**							**±4 d**	**±14 d**	
Solicited AE recording		- - - - - *continuous* - - - - -											❺
Unsolicited AE recording [q]	- *continuous* -												●
SAE recording	- *continuous* -												●
Concomitant medications [s]	- *continuous* -												●
Challenge virus inoculation							●						
Nasal wash sample [t]					●				❽	●			
RVAT (from nasopharyngeal swab) [c]										●			
Subject symptom card					●		❼	❼	❼	●			
Spirometry	●				●		●	●	●	●			
24-hour tissue count and mucus weight					●		●	●	●	●			
Approx daily blood draw, mL	10	70	–	10	70		–	–	30	10	70	–	70
Approx cumulative study blood draw, mL	10	80	80	90	160		160	160	190	200	270	**270**	–

AE = adverse event; d = day; chall = challenge; ECG = electrocardiogram; HIV = human immunodeficiency virus; ICF = informed consent form; mo = month; RVAT = rapid virus antigen test; SAE = serious adverse event; vacc = vaccination

❶ pre-dose; ❷ pre- and post-dose; ❸ screening laboratory tests are to be done within 56 days of randomization; ❹ blood samples for immunogenicity will only be taken if the early exit is ≥14 days after the previous immunogenicity blood draw; ❺ if within 7 days of study vaccination; ❻ check of diary during the telephone call; ❼ three times daily; ❽ every 12 (±1) hours; ❾ on Days 3, 7 and 11; ❿ Days 3, 7, and 11 for cardiac enzymes only. *Note*: cardiac enzymes will also be assessed on entry to the quarantine Unit in addition to biochemistry and hematology parameters.

a. Intranasal challenge with the RSV-A Memphis 37b virus will occur on Day 0, not less than 24 or more than 90 days after vaccination.
b. At 48-hours post-vaccination, a telephone call will additionally be made to two sentinel subjects to collect safety information.
c. Subjects will only be discharged if no detectable virus is present (negative RVAT) in nasopharyngeal samples taken on Day 12. If symptoms are present, but no virus is detected (negative RVAT), discharge will be at the investigator's discretion. If appropriate, subjects may reside in quarantine for an additional night or longer before discharge.
d. Historical pre-screening data collected through the ethics committee-approved hVIVO screening protocol within 56 days (90 days for viral serology) to 3 days prior to vaccination. may be used for screening procedures. Historical pre-screening data obtained prior to this window can be re-assessed any time from 40 days to 3 days prior to vaccination.
e. For those subjects who are unable to continue participation in the study, but for whom consent is not withdrawn, an exit visit will be conducted as soon as possible.
f. ie, 56 days to 3 days prior to vaccination.
g. By telephone.
h. Study-specific consent may occur on the day of vaccination, providing all required eligibility information has been collected through the ethics committee-approved hVIVO screening protocol.
i. Directed physical examination includes respiratory system, nose, ear, throat, and facial lymph nodes examination.
j. Supine systolic and diastolic blood pressure, heart rate and respiratory rate after at least 5 minutes rest.
k. Supine ECG after at least 5 minutes rest.
l. Neutralizing antibody to RSV-A Memphis 37b; blood will be collected within 90 days of vaccination and on entry to the Unit.

m. For all female subjects.
n. To include exclusion criteria 1, 7, 8, 9, 10, 14 and 16.
o. The investigator must check for acute illness or body temperature ≥37.8 °C at the time of vaccination. In such cases, the subject may be re-screened (if vaccination number not assigned), or withdrawn at the discretion of the investigator. *Note*: the minimum time between vaccination and challenge is 24 days.
p. Subjects will be closely observed for a minimum of 30 minutes post-vaccination. Any unsolicited, solicited local and systemic AEs, and vital signs will be documented by study-site personnel following this observation period.
q. Unsolicited AEs will be collected from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge.
r. Rulers and thermometers will be distributed at the vaccination visit.
s. Concomitant therapies will be collected and recorded in the eCRF from time of study vaccine administration until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge, and additionally outside of these periods when associated with an SAE.
t. Aliquots may be taken from nasal wash samples if more than one assay is to be assessed at a given timepoint.

ABBREVIATIONS

Ad26	adenovirus serotype 26
Ad35	adenovirus serotype 35
AE	adverse event
AUC	area under the curve
β-hCG	β-human chorionic gonadotropin
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CS	circumsporozoite
DNA	deoxyribonucleic acid
DRC	Data Review Committee
ECG	electrocardiogram
eCRF	electronic case report form
eDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot (assay)
ERD	enhanced respiratory disease
FA	full analysis
F protein	fusion protein
FDA	(US) Food and Drug Administration
FI	formalin-inactivated
FIH	first-in-human
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IEC	Independent Ethics Committee
IFNγ	interferon gamma
IRB	Institutional Review Board
ITTc	intent-to-treat-challenge
PPI	per-protocol immunogenicity
PQC	Product Quality Complaint
RVAT	rapid virus antigen test
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase-polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SOP	standard operating procedure
SRP	study-responsible physician
SSC	subject symptom card
SUSAR	suspected unexpected serious adverse reaction
Th	T-helper (cell)
TNFα	tumor necrosis factor alpha
US	United States
VL-AUC	AUC for viral load
vp	viral particles
WBC	white blood cell

1. INTRODUCTION

A human adenovirus-vectored vaccine candidate which has shown promise in preclinical animal models of respiratory syncytial virus (RSV), and which is currently being tested in elderly subjects aged ≥60 years in stable health in Study VAC18193RSV1003, will be assessed in this study:

- Ad26.RSV.preF (JNJ-64400141), a replication-incompetent adenovirus serotype 26 (Ad26) containing a deoxyribonucleic acid (DNA) transgene that encodes for the pre-fusion conformation-stabilized F protein (pre-F) derived from the RSV A2 strain.

This will be an exploratory Phase 2a study where the prophylactic efficacy of Ad26.RSV.preF in the human challenge model of RSV infection will be assessed, and the appropriateness of the RSV human challenge model for RSV vaccine development will be evaluated on the basis of its ability to show a trend toward prophylactic efficacy with a RSV vaccine that has shown promise in preclinical challenge models.

For the most comprehensive nonclinical and clinical information regarding Ad26.RSV.preF, refer to the latest version of the Investigator's Brochure for Ad26.RSV.preF.[1]

The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document.

1.1. Background

RSV is considered to be the most important cause of serious acute respiratory illness in infants and children under 5 years of age.[16,34,38] Globally, in 2005, RSV was responsible for an estimated 3.4 million hospitalizations worldwide in children under 5 years of age. Furthermore, 66,000 to 199,000 children younger than 5 years died from RSV-associated acute lower respiratory tract infection (LRTI) in 2005, and 99% of these deaths occurred in developing countries.[27] Nevertheless, the disease burden due to RSV in developed countries is substantial, with RSV infection during childhood linked to the development of wheezing, airway hyperreactivity and asthma.[31,33,35,36,37] In the United States (US), the infection rate was 68.8% in children younger than 12 months of age and 82.6% during the second year of life. Virtually all children had been infected at least once by 24 months of age, and about one half had experienced 2 infections.[14] In the US, RSV infection in children under 5 years of age is the cause of 57,000 to 175,000 hospitalizations, 500,000 emergency room visits, and approximately 500 deaths each year.[30,34,38] In children under 1 year of age, RSV is the most important cause of bronchiolitis, and RSV hospitalization is highest among children under 6 months of age.[6,16]

In addition to children, RSV is an important cause of respiratory infections in the elderly, immunocompromised, and those with underlying chronic cardiopulmonary conditions.[10] In long-term care facilities, RSV is estimated to infect 5-10% of the residents per year with significant rates of pneumonia (10 to 20%) and death (2 to 5%).[11] In one epidemiology study of RSV burden, it was estimated that 11,000 elderly persons die annually of RSV in the US.[39] These data support the importance of developing an effective vaccine for certain adult populations.

Despite the high disease burden, no licensed vaccine is available for RSV. The first vaccine candidate for young children, which consisted of formalin-inactivated RSV (FI-RSV), was associated with enhanced respiratory disease (ERD) upon infection with RSV.[19] Although the mechanisms for ERD are not fully understood, it is thought that FI-RSV failed to induce adequate neutralizing antibody titers and CD8 priming, and induced a T-helper (Th) 2 skewed response.[26]

Adenoviral-vectored Vaccines

It is thought that an efficacious RSV vaccine should induce high levels of neutralizing antibodies, antigen-specific $CD8^+$ T-cell responses, and Th1-type $CD4^+$ T cells.[3] The candidate RSV vaccine being evaluated in this protocol is based on the AdVac® platform which has been shown to promote a strong antibody response, as well as $CD8^+$ T cell and Th1-type $CD4^+$ T-cell responses.

The immunogenicity profile of adenoviral vectors, with particular emphasis on Th1 responses, is illustrated by data obtained from immunization of adults with Ad26-vectored human immunodeficiency virus (HIV) vaccine (Ad26.ENVA.01), and of adults and infants with an adenovirus serotype 35 (Ad35)-vectored tuberculosis (TB) vaccine (Ad35.TB-S). These data show predominantly interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) production in $CD4^+$ and $CD8^+$ T cells.[2,5,29] Furthermore, in mice, Ad26- and Ad35-vectored vaccines with malaria circumsporozoite (CS) transgene inserts (Ad26.CS.01 and Ad35.CS.01), when administered as single immunizations or in combination as a heterologous prime-boost regimen at dose levels ranging from $1x10^8$ viral particles (vp) to $1x10^{10}$ vp, induce predominantly $CD8^+$ T-cell responses, as well as mainly immunoglobulin (Ig) G2a antibody responses, indicative of a Th1-biased response.[32]

Ad26.RSV.FA2 Clinical Data

Ad26 encoding for the wild-type RSV FA2, Ad26.RSV.FA2, has been evaluated in studies VAC18192RSV1001 and VAC18192RSV1003 (N = 48 and 32, respectively, of which 35 and 24 subjects, respectively, received Ad26.RSV.FA2) in adults at doses of $5x10^{10}$ vp. All subject visits have been completed in both studies.

Results indicate that there have been no safety concerns following vaccination in either study. After vaccination with Ad26.RSV.FA2, local reactogenicity comprised almost exclusively mild to moderate pain of median duration 1 to 3 days. The most commonly experienced solicited systemic adverse events (AEs; headache, fatigue, chills and myalgia) were also mostly mild to moderate in severity and of median duration 1 to 3 days; most unsolicited AEs and most laboratory abnormalities were mild to moderate in severity. No serious adverse events (SAEs) were reported and no AEs led to withdrawal from study vaccine.

Single vaccination with $5x10^{10}$ vp Ad26.RSV.FA2 raised humoral and cellular immunity; humoral and cellular immune responses were maintained for at least 6 months following

vaccination. An increase in RSV neutralizing antibody titers was observed; RSV-specific T-cell responses were also increased.

FA2 and preF RSV Vaccines

The candidate vaccine assessed in this study is Ad26.RSV.preF, ie, a replication-incompetent Ad26-containing a DNA transgene that encodes for the pre-fusion conformation-stabilized F protein derived from the RSV A2 strain.

First-in-human (FIH) clinical studies (VAC18192RSV1001 [FIH for Ad35.RSV.FA2] and VAC18192RSV1003 [FIH for Ad26.RSV.FA2]) have been completed with vaccines Ad26.RSV.FA2 and Ad35.RSV.FA2 (ie, a similar recombinant, replication-incompetent vaccine using an Ad35 vector), in which Ad26 and Ad35, respectively, encode for a wild-type RSV F protein of the RSV A2 strain.

The adenoviral vectors Ad26 and Ad35 are derived from Group B and D serotype adenoviruses and have been similarly modified to be replication-incompetent; expression of the antigen is controlled by the same promotor. An Ad26-based RSV vaccine was chosen for further clinical development over the Ad35-based counterpart based on a better immunogenicity profile from nonclinical data, a similar safety and immunogenicity profile but at half the Ad35 dose from clinical data, and a better manufacturing profile.

The F protein of RSV F undergoes a conformational transition from a metastable pre-fusion conformation to a stable post-fusion conformation. Neutralizing sensitive epitopes reside on both proteins, but recent evidence indicates that those epitopes specific to the pre-F protein seem to be more potent than those previously identified and present on the post-F protein.[13,15] This evidence in the design of the candidate RSV vaccine (Ad26.RSV.preF) in which the adenoviral vector encodes for a full length RSV F protein stabilized in the pre-F protein conformation. CCI [redacted] [22] This change in the transgene confers more stability to the pre-fusion form of the protein.[22] This change also induces higher immune responses against pre-fusion epitopes because the majority of neutralizing antibodies target the pre-fusion protein conformation.[23,28] For these reasons, it is anticipated that the Ad26.RSV.preF vaccine candidate will generate more neutralizing antibodies relative to the Ad26.RSV.FA2 vaccine.[42]

Ad26.RSV.preF Preclinical Data

Ad26.RSV.preF is immunogenic in mice and cotton rats, with humoral responses that include the induction of RSV neutralizing antibodies. In addition, in mice it was shown that Ad26.RSV.preF elicits cellular responses, characterized by the induction of RSV F-specific $CD8^+$ $IFN\gamma^+$ T cells. The immune response after Ad26.RSV.preF immunization was Th1 biased. In cotton rats, single immunization with Ad26.RSV.preF protects the upper and lower respiratory tract from challenge with RSV A2 and RSV B strains, without induction of any histopathological signs of ERD.[42]

Ad26.RSV.preF Clinical Data

Ad26.RSV.preF is under evaluation in the ongoing FIH Phase 1 study VAC18193RSV1003 in elderly subjects aged ≥60 years in stable health. In this randomized, placebo-controlled, double-blind study, 72 elderly male and female subjects have been randomized in parallel to one of five study groups and have received the first (Day 1) of two intramuscular injections as follows:

- Group 1 – $5x10^{10}$ vp Ad26.RSV.preF on Day 1 and 1 year later;
- Group 2 – $5x10^{10}$ vp Ad26.RSV.preF on Day 1 and placebo 1 year later;
- Group 3 – $1x10^{11}$ vp Ad26.RSV.preF on Day 1 and 1 year later;
- Group 4 – $1x10^{11}$ vp Ad26.RSV.preF on Day 1 and placebo 1 year later;
- Group 5 – placebo on Day 1 and 1 year later.

Safety and immunogenicity data from the unblinded interim analysis 28 days post-Dose 1 from all 72 subjects who received Ad26.RSV.preF ($5x10^{10}$ vp or $1x10^{11}$ vp) or placebo confirmed the immunogenicity of the vaccine; the $1x10^{11}$ vp dose of Ad26.RSV.preF was more immunogenic than the $5x10^{10}$ vp dose. No safety concerns were revealed; the reactogenicity of both doses was comparable.

For study VAC18193RSV1003, Ad26.RSV.preF was provided in a different buffer (Formulation Buffer 1[a]) from the one to be used in the current study (Formulation Buffer 2[b]). The formulation buffer was changed to enhance the Ad26.RSV.preF drug product stability at 2 to 8 °C for storage purposes in future studies. The new formulation buffer was considered well-tolerated when tested in a nonclinical single-dose intramuscular safety study in rabbits.[42]

Safety Data Supporting Dose Selection from Other Ad26-based Vaccines

In addition to the two completed studies with Ad26.RSV.FA2 and one ongoing study with Ad26.RSV.preF, the dose level for Ad26.RSV.preF used in this study is supported by experience in adults with other Ad26 vaccines encoding for different antigens (EnvA [in Ad26.ENVA.01 against HIV];[4,5] CS protein [in Ad26.CS.01 against malaria];[7,29] and Ebola glycoprotein [in Ad26.ZEBOV against Ebola virus][25]). Note that, in general, at a given dose level, no significant changes in the safety profiles of Ad26-based vaccines have been seen when the transgene has been changed.

In completed clinical studies,[c] the safety of Ad26.ENVA.01, Ad26.CS.01 and Ad26.RSV.FA2 has previously been evaluated in at least 293 adult subjects, of whom 243 have received

a CCI

b [redacted]

[c] Safety data from these studies are summarized in a separate report (Adenoviral Vaccine Safety Database V2.0, December 2016) which is available on request.

$5x10^{10}$ vp, and found to be well-tolerated. In addition, four Phase 1 studies with Ad26.ZEBOV have been completed in adults: 291 subjects have received Ad26.ZEBOV at $5x10^{10}$ vp and $1x10^{11}$ vp without significant safety issues: 15 subjects have received $1x10^{11}$ vp (25 doses administered), and 276 subjects have received $5x10^{10}$ vp. The safety data from these Ebola studies showed no safety concerns at $5x10^{10}$ vp and $1x10^{11}$ vp. Overall, these clinical data are supportive of dosing Ad26.RSV.preF at $1x10^{11}$ vp in the current study.

1.2. Overall Rationale for the Study

This is an exploratory, double-blind, randomized, placebo-controlled human challenge study in which healthy adult subjects will receive single intramuscular doses of $1x10^{11}$ vp of Ad26.RSV.preF or placebo and 28 days later (window between 24 to 90 days) will undergo intranasal challenge with RSV-A Memphis 37b virus. This is an exploratory study as an RSV vaccine has not previously been tested in this challenge model. The prophylactic antiviral effect, prevention of clinical symptoms of RSV infection, and prevention of RSV infection will be evaluated. The study will also explore the relationship between immunogenicity and efficacy outcomes.

The study is considered to be exploratory because no RSV vaccine has been previously tested in the human challenge model of RSV infection. The RSV vaccine developed by the sponsor is mainly intended to protect against moderate-severe low respiratory tract RSV infection. The RSV symptoms seen in this challenge model are mild and mainly limited to the upper respiratory tract compared to those which would be observed in a clinical efficacy trial of naturally-occurring infection where more severe symptoms, plus RT-PCR-confirmed RSV infection, would be measured (see Attachment 4). Although it might be expected that a vaccine would be better able to protect against mild disease than severe disease, it might also be that mild upper respiratory disease could be more difficult for a vaccine to protect against, for the reason that a parenteral vaccine would induce lower levels of antibody and cellular responses in the nasopharynx compared to levels induced in the blood and lower respiratory tract. Preclinical data indicates this might be the case: when the dose of vaccine is reduced, protection against virus infection in the nasopharynx is lost before protection in the lung. It is not known whether the vaccine in this study, which is to be dosed at the highest dose already given safely to humans, will induce a sufficient immune response to give protection in the nasopharynx, or at least to give an indication of some protection. Therefore this study is considered highly exploratory, and is as much a test of whether this model will prove to be useful in vaccine studies, as it is a study of the effectiveness the RSV vaccine under development.

Since preclinical models have suggested some level of protection by the vaccine against nasopharyngeal infection, the study includes a formal primary objective of lowering area under the curve of viral load (VL-AUC), despite the exploratory nature of this study. This VL-AUC has been found in previous studies to be the most sensitive and least variable parameter for efficacy measurement in this model. If the vaccine can prevent infection completely in some subjects, a powerful effect on the AUC would be seen since in those subjects the AUC would be 0. There are also a small number of secondary objectives related to reduction of symptoms

occurring at a specific time, reduction of overall mucous secretion and incidence of infection utilizing more severe or very mild symptoms as alternative definitions, coupled in both cases with two quantifiable RT-PCR measurements to confirm the symptoms are due to RSV.

The statistical approach to this exploratory study is to have an alpha of 20% to indicate a trend and 5% as a significant result for the primary endpoint. If positive results or a trend in the primary or secondary objectives are found, this would suggest that the challenge model may be a reasonable approach to examine vaccine efficacy and would provide evidence to perform more extensive studies. If this study fails its primary endpoint, and does not show any trends in the other endpoints, it would suggest that the human RSV challenge model is not an appropriate model to evaluate potential efficacy of RSV vaccines directed against more serious disease that has more of a lower respiratory component.

Therefore this study will serve as a pilot to explore the value of evaluating RSV vaccines in the human challenge model in RSV vaccine development, with the possibility that, if the model is appropriate, a trend toward efficacy with Ad26.RSV.preF could be detected.

1.3. Risk/Benefit Section

1.3.1. Known Benefits

The clinical benefits of vaccination with Ad26.RSV.preF have yet to be established. For the subjects in this study the vaccine may have no direct benefit.

1.3.2. Potential Benefits

Results from clinical studies with Ad26.RSV.preF may be useful in developing a new vaccine to prevent RSV infection. The vaccination may provide some protection against subsequent RSV infection in adults participating in the study who have been screened for susceptibility to RSV infection.

1.3.3. Known Risks

All vaccines have the potential to cause adverse experiences. To date, limited clinical data with Ad26.RSV.preF are available from the Phase 1 VAC18193RSV1003 study in elderly subjects aged ≥60 years in stable health. Safety data from an unblinded interim analysis at 28 days post-Dose 1 from all subjects who received Ad26.RSV.preF ($5x10^{10}$ vp or $1x10^{11}$ vp) or placebo did not reveal any safety concerns.

No specific safety concerns were identified based on the currently available clinical data.

1.3.4. Potential Risks

The following potential risks for Ad26.RSV.preF will be monitored during the study.

Risks Related to Vaccines

Subjects may exhibit local signs and symptoms associated with vaccination, including erythema, swelling/induration, and pain/tenderness. These local reactions will be monitored, but are generally short-term and do not require treatment.

Subjects may exhibit general signs and symptoms associated with administration of a vaccine, or vaccination with placebo, including fatigue, headache, myalgia, arthralgia, chills and nausea. These side effects will be monitored, but are generally short-term and do not require treatment.

Subjects may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions, including anaphylaxis, are rare but can occur with any vaccine. Medications will be available in the clinic to treat serious allergic reactions promptly. Subjects with a known allergy, or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine components (including any of the constituents of the study vaccine) will be excluded from the study. The study site should have medical treatment available in case of severe allergic reactions following vaccine administration.

Risks Related to Adenoviral-vectored Vaccines

Safety data available from 10 completed clinical studies in adults with other Ad26-vectored vaccine candidates, in which Ad26 with different inserts has been evaluated at dose levels ranging from $1x10^9$ vp to $1x10^{11}$ vp, indicate that no safety concerns would be anticipated from vaccination with Ad26.RSV.preF at doses of $5x10^{10}$ vp and $1x10^{11}$ vp.

Local AEs (moderate injection site pain and tenderness, and moderate to severe redness at the injection site) and systemic AEs (headache, chills, joint pain, muscle pain, tiredness/generally not feeling well/fatigue and fever) have been reported after vaccination with Ad26-vectored vaccines. In a few subjects, transient laboratory abnormalities have been seen, including changes in neutrophils (neutropenia/neutrophil count decreased). Laboratory changes including decreased hemoglobin, decreased platelets, and moderate elevations in liver transaminases were observed that were not associated with any clinical findings and appear to be transient based on no reported persistent abnormalities in any of the subjects.

For further details on the safety profiles of other Ad26-vectored vaccine candidates, see the Ad26.RSV.preF Investigator's Brochure.[1]

Risks from Collection of Nasal Wash Samples/Nasopharyngeal Swabs

Collection of nasal wash samples or nasopharyngeal swabs may cause a nosebleed.

Risks from Blood Draws

Blood drawing may cause pain/tenderness, bruising, bleeding, lightheadedness, dizziness, vasovagal response, and, rarely, infection at the site where the blood is taken.

Pregnancy and Birth Control

The effect of the study vaccine on a fetus or nursing baby is unknown so women of childbearing potential are required to agree to practice effective birth control measures for sexual intercourse from signing the informed consent form (ICF) until at least 4 months after vaccination (see Section 4.1). Use of hormonal contraception should start at least 28 days before vaccination. Women who are pregnant or breast-feeding, or are planning to become pregnant while enrolled in the study until 6 months after study vaccination, will be excluded from the study. Men having sexual intercourse with women or men are required to agree to use effective contraception for sexual intercourse starting from entry to the quarantine Unit and continuing until 3 months after the date of viral challenge (see Section 4.3).

Subjects with Immuno-suppression/Reduced Immune Response

Limited evidence indicates that inactivated vaccines (or non-replicating viral vaccines) generally have the same safety profile in immunocompromised patients as in immunocompetent individuals. However, the magnitude, breadth, and persistence of the immune response to vaccination may be reduced or absent in immunocompromised persons. Subjects with abnormal function of the immune system will be excluded from the study.

Concomitant Vaccination

Concomitant vaccination might have an influence on both safety profile and immunogenicity of Ad26.RSV.preF. Likewise, Ad26.RSV.preF might have an influence on both safety profile and immunogenicity of any concomitant vaccination. As a result, vaccination with live attenuated vaccines within 28 days of a study vaccination (ie, before and after) is prohibited. Other vaccines (eg, influenza, tetanus, hepatitis A, hepatitis B, rabies) should be given at least 14 days before (or at least 14 days after) administration of study vaccine. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine.

Risks Associated with the Human Challenge Model

Healthy adult subjects will be recruited as they are best suited to tolerate inoculation of the virus.

Severe complications tend to occur almost exclusively in infants, the elderly, and persons of any age with chronic co-morbidities or who are significantly immune-compromised. Qualified doctors and nurses in the quarantine Unit will manage any symptoms.

As a consequence of the RSV-A Memphis 37b inoculation, AEs (serious or non-serious) related to RSV infection commonly occur. These anticipated AEs are related to, but not limited to, rhinitis, rhinorrhea, nasal congestion, sneezing, nasopharyngitis, ear ache, malaise (tiredness), cough, shortness of breath, muscle and/or joint pain, headache, fever, nasal blood spotting, and

upper respiratory tract infection. In addition, laboratory abnormalities related to RSV viral infection may occur such as, but not limited to, hematologic changes (increases in white blood cell [WBC] count and changes in differential values) and increased levels of inflammatory markers (eg. C-reactive protein).

The study virus, like many viruses, can cause more substantial health issues such as myocarditis. However, the chance of this resulting in permanent changes is rare as most cases are minor and resolve without sequelae. In over 500 subjects inoculated with the study virus by hVIVO, clinical laboratory tests in 2 subjects have shown changes suggestive of myocardial inflammation: both cases returned to normal without treatment and were not associated with specific symptoms or ECG changes.

It is unlikely that subjects will transmit RSV virus to their close contacts after they have been released from the quarantine Unit. After infection with RSV, infectious virus will be present in the subject's nose for several days, but it is not expected to be present in the subject's nose at the time of discharge from the Unit. This is because the usual duration of time that RSV remains infective in adults is several days shorter than the time they will spend in the Unit in isolation.[9]

All subjects will stay in the quarantine Unit from 1 or 2 days prior to RSV inoculation until 12 days after challenge. Nasopharyngeal swabs will be tested for the presence of virus prior to discharge using rapid virus antigen test (RVAT). Subjects will only be discharged when no detectable virus is present (negative RVAT). If symptoms are present, but no virus is detected (negative RVAT), discharge will be at the investigator's discretion. If appropriate, subjects may reside in quarantine for an additional night or longer before discharge.

To reduce the risk of passing RSV on to others, the subject will be advised to avoid contact with the following vulnerable groups of people for two weeks after they leave the quarantine Unit:

- any person with any known immunodeficiency
- any person receiving immunosuppressant medications
- any person undergoing or soon to undergo cancer chemotherapy
- any person who has congestive heart failure or is in frail health
- any person who has a diagnosis of emphysema or chronic obstructive pulmonary disease (COPD), is elderly residing in a nursing home, or has severe lung disease
- any person who has received a transplant (bone marrow or solid organ)
- children below the age of 1 year
- elderly persons (aged 75 years or older).

Unknown Risks

There may be other serious risks that are not known.

1.3.5. Overall Benefit/Risk Assessment

Based on the available data and proposed safety measures, the overall benefit/risk assessment for this clinical study is considered acceptable for the following reasons:

- The dose of Ad26.RSV.preF to be used in this study will be $1x10^{11}$ vp. There were no safety concerns with this dose in study VAC18193RSV1003 in elderly subjects aged ≥60 years in stable health. This dose was found to be safe and well-tolerated in other Ad26-vectored vaccines against HIV and Ebola.
- The study will be paused prior to challenge if one or more subjects experience any vaccine-related SAEs, or two or more subjects experience severe AEs considered to be at least possibly related to Ad26.RSV.preF after discussion between sponsor and investigator (see Section 17.9.2). In these situations, the pausing rules will apply (see Section 11.10).
- The study will be terminated if one or more subjects experience any severe or clinically significant illness from RSV challenge, after discussion with the investigator, hVIVO, and the sponsor's study-responsible physician (SRP) (see Section 17.9.2).
- Several safety measures have been proposed to minimize potential risk to subjects, including:
  - Only subjects who meet all of the inclusion criteria and none of the exclusion criteria (as specified in the protocol) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of subjects in the study.
  - Utilization of sentinel subjects (see Section 3.1).
  - Utilization of withdrawal criteria (see Section 10.2).
  - Close monitoring of subjects throughout the study as described in Section 9.

2. OBJECTIVES, ENDPOINTS, AND HYPOTHESIS

2.1. Objectives and Endpoints

2.1.1. Objectives

Primary Objective

The primary objective is to assess a trend for the prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly to adults aged 18-50 years in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the area under the curve (AUC) over time by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) compared to placebo.

Secondary Objectives

- To assess a trend for prophylactic efficacy of a single dose of Ad26.RSV.preF in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the peak viral load of the RT-PCR compared to placebo.
- To assess the effect of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF on viral load, as measured by RT-PCR and quantitative culture of RSV, and clinical symptoms on Day 6 and Day 7 post-challenge compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two quantifiable RT-PCR measurements and one or more positive clinical symptoms of any grade from two different categories in the symptom scoring system (Upper Respiratory, Lower Respiratory, Systemic), or one Grade 2 symptom from any category compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two quantifiable RT-PCR measurements and one or more positive clinical symptoms of any grade in any category from the symptom scoring system compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of the weight of mucous secretions and tissue count over time compared to placebo.
- To assess the safety and tolerability of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly.

Exploratory Objectives

- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of clinical symptoms as measured by the AUC over time of symptoms collected by a graded symptom scoring system compared to placebo.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of AUC for viral load (VL-AUC), as measured by quantitative culture of RSV from nasal wash samples, compared to placebo.
- To explore the relationship of humoral (including nasal wash samples if feasible) and cellular immunogenicity outcomes and the VL-AUC and the AUC for clinical symptoms.
- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection defined as two quantifiable RT-PCR measurements and/or positive serological measurement of RSV infection in the context of immunization and pre-existing antibody and one or more positive clinical symptoms of any grade in any category compared to placebo.

- To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of respiratory parameters measured by spirometry during the study compared to placebo.
- To explore the relationships between clinical symptoms and viral load, as measured by RT-PCR and quantitative culture of RSV, in Ad26.RSV.preF subjects and placebo.
- To explore other immunologic parameters in relation to the VL-AUC by RT-PCR and AUC for clinical symptoms and occurrence of symptomatic RSV infection defined as two quantifiable RT-PCR measurements or more plus one clinical symptom of any grade.
- To explore the immune response of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF compared to placebo.
- To explore humoral and cellular responses to challenge with RSV-A Memphis 37b in immunized and non-immunized subjects.
- To explore the relationship between immunogenicity and pre-existing Ad26 neutralizing antibody.
- To explore other potential efficacy endpoints, as defined in the statistical analysis plan (SAP), to determine the best efficacy endpoint to use in subsequent clinical studies investigating the vaccine's efficacy.

Objectives and endpoints are tabulated in Attachment 2.

2.1.2. Endpoints

Primary Endpoint: Viral Load

The primary endpoint is the VL-AUC of RSV-A Memphis 37b as determined by quantitative RT-PCR assay of nasal wash samples.

Nasal wash samples are taken every 12 (±1) hours beginning two days (ie, in the morning) after inoculation of the challenge virus. Quantitative RT-PCR is utilized to measure viral load.

Secondary Endpoints

- Peak viral load of RSV-A Memphis 37b, defined as the maximum viral load as determined by quantitative RT-PCR assay of nasal wash samples, observed over the entire time period.
- Occurrence of symptomatic RSV infection defined as two quantifiable RT-PCR measurements at different timepoints plus symptoms of any grade from two different categories from the subject symptom card (SSC)[a] or two quantifiable RT-PCR measurements plus any Grade 2 symptom from any category. The three SSC categories are:
  - Upper Respiratory symptoms: runny nose, stuffy nose, sneezing, sore throat, earache

[a] See Attachment 3.

  - Lower Respiratory symptoms: cough, shortness of breath, chest tightness, wheeze
  - Systemic symptoms: malaise, headache, muscle and/or joint ache, chilliness/ feverishness

- Occurrence of RSV infection defined as two quantifiable RT-PCR measurements plus any clinical symptom of any severity.
- Total weight of mucus produced and tissue count.
- Safety and tolerability
  - Unsolicited adverse events (AEs) from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge.
  - Safety data including, but not limited to, physical examinations, vital signs, 12-lead electrocardiograms (ECGs) and clinical laboratory results (including biochemistry, hematology, and urinalysis).
  - Serious adverse events (SAEs) throughout the study (from signing the ICF to the end of the study, 6 months after vaccination).
  - Solicited local and systemic AEs for 7 days after vaccination.

Exploratory Endpoints

- AUC of the total clinical symptom score. The total clinical symptom score is a composite of 13 self-reported symptoms (on the SSC) divided into three categories (Upper Respiratory, Lower Respiratory, Systemic).
- The VL-AUC of RSV-A Memphis 37b, as determined by quantitative culture of RSV of nasal wash samples.
- Pre-F antibody in nasal wash samples measured by pre-F enzyme-linked immunosorbent assay (ELISA) normalized to a standard protein or antibody to account for variability of sample volume, dilution and concentration in nasal wash samples.
- Proportion of subjects with symptomatic RSV infection (RT-PCR or serology). Seroconversion is defined as a four-fold rise against the G and/or N protein by ELISA which would indicate a take of the virus inoculum to such extent that a systemic immunologic response against an antigen not in the vaccine was induced.
- Neutralizing antibody against RSV-Memphis 37b (challenge strain) and RSV A2.
- RSV pre-F and post-F antibodies from serum and nasal wash samples[a] measured by ELISA.
- Respiratory parameters: spirometry (FEV1, FVC, FEV1/FVC).

[a] Assumes that the ELISA for nasal wash samples will be available.

- Vaccine immune response defined as specific pre-F levels of antibody as measured by ELISA, specific neutralizing antibody levels, F antigen-specific cellular responses as measured by interferon gamma enzyme-linked immunospot (IFNγ ELISpot) assay, flow cytometry cytokine analysis and/or secreted cytokines compared to placebo.

Additional exploratory analyses may be performed to investigate vaccine-elicited immune responses further. These may include, but are not limited to, the following assays:

- RSV cross-neutralization of B and/or other A strain
- Binding antibodies to RSV G and/or N
- F-protein antibody specificity characterization
- Adenovirus neutralization assay
- Functional and molecular antibody characterization

Refer to Section 9.2 for evaluations related to endpoints.

Objectives and endpoints are tabulated in Attachment 2.

2.2. Hypothesis

This is an exploratory human challenge study that will evaluate the effect of Ad26.RSV.preF on viral replication and on clinical symptoms of RSV infection after challenge. To focus interpretation, a formal primary hypothesis is described that will be statistically tested. The primary hypothesis is that a single dose of $1x10^{11}$ vp of Ad26.RSV.preF shows a trend of reduction in VL-AUC of the quantitative RT-PCR in healthy subjects challenged intranasally with the RSV-A Memphis 37b virus, compared with healthy subjects given placebo who are similarly challenged.

As no RSV vaccine has been previously tested in a human challenge model, the statistical results of this hypothesis test will need to be interpreted with care as the relation between the results from this human challenge model and vaccine efficacy is unknown. An effect that is significant at 5% (one-sided) will be considered a significant effect. An effect that is significant at 20% (one-sided) will be considered as a trend. This trend, if observed, should be confirmed in future studies.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

This is a single center, randomized, placebo-controlled, double-blind Phase 2a human challenge study, to be conducted in approximately 44 healthy male and female subjects aged 18-50 years

who have been pre-screened for susceptibility to RSV infection, ie, have levels of RSV neutralizing antibodies compatible with susceptibility to RSV infection.[a]

Subjects will receive single intramuscular doses of $1x10^{11}$ vp of Ad26.RSV.preF or placebo. More than 44 (and up to 70) subjects will be vaccinated to account for withdrawals between vaccination and challenge. The challenge study site, hVIVO, has the capacity to challenge and house under isolation 22 subjects at a time and thus the study will be conducted in several cohorts with up to 22 subjects per cohort. Within each cohort, subjects will be randomized 1:1 to $1x10^{11}$ vp of Ad26.RSV.preF or placebo.

Table 4: Study Design: Vaccination and Challenge

Group	N	Day –28	Day 0*
1	22	Ad26.RSV.preF ($1x10^{11}$ vp)	Challenge with RSV-A Memphis 37b**
2	22	Placebo	

* ie, not less than 24 or more than 90 days after vaccination.

**Subjects will be challenged in two or more cohorts of up to 22 subjects per cohort. Within each cohort, subjects will be randomized 1:1 to $1x10^{11}$ vp of Ad26.RSV.preF or placebo.

Note: The infection rate (based on mucus weight, symptoms and viral shedding) in the placebo group will be followed on an ongoing basis by an unblinded individual who does not have any other study function to ensure a sufficient infection rate has occurred. If the infection rate is lower than anticipated, additional subjects may be enrolled, initially four additional subjects in each arm and up to 70 in total. This is to ensure that a high enough number of evaluable subjects is reached in each arm.

Initially, two sentinel subjects will be enrolled and vaccinated, and 48-hour safety will be checked by the investigator/SRP after vaccination. Safety data for review will include solicited and unsolicited AEs and SAEs. In the absence of significant findings, the rest of the subjects will be enrolled and vaccinated. The sentinel approach with 2 subjects (1 active and 1 placebo) is taken to ensure active and placebo subjects are included in the evaluations, taking into account the blinding. The observation at 48 hours post-dose is primarily to rule out anaphylaxis or cytokine storm.

All subjects will be closely observed for a minimum of 30 minutes post-vaccination, to monitor for the development of any acute reactions, or longer if deemed necessary by the investigator. Any unsolicited, solicited local or systemic AEs and vital signs will be documented by study-site personnel following this observation period. Subjects will be given a thermometer, ruler and daily assessment (subject) diary with instructions for the proper recording of events. Each subject will record solicited local (at injection site) and systemic AEs and body temperatures, beginning on the evening of the study vaccine dosing day and on a daily basis for the following 7 days. Body temperatures should be taken at approximately the same time each day, preferably in the

[a] The cut-off is based on the average 25th percentile of the past 12 months screening results.

evening.[a] Study-site personnel will collect and review subject diary information and confirm the entries at subsequent site visits.

One to two days prior to viral challenge, subjects will be admitted to the Unit to confirm eligibility for challenge and to orient them to the challenge Unit. At this time, assessments will include relevant medical information since vaccination, physical examination, ECG and clinical laboratory testing. Blood will be collected for immunogenicity assessments. The target day for intranasal challenge with the RSV-A Memphis 37b virus is Day 0, 28 days after vaccination, with a window of 24 days to 90 days after vaccination. The 90 day window is to account for the few "overflow" subjects that were vaccinated (to be available to replace any withdrawals) but may not have been challenged but might be available for a subsequent challenge cohort as explained in more detail below.

This range for timing of the challenge is specified for the following reason. A total of 26 subjects will be vaccinated for the first challenge cohort, of whom the first 22 who are vaccinated will be challenged. Any subjects that are vaccinated but not challenged in the first cohort ("overflow" subjects) will be included in a subsequent cohort if they are still available and continue to meet all entry criteria. The withdrawal rate between vaccination and challenge for the first cohort will be used to assess how many extra subjects will need to be vaccinated for subsequent cohorts.[b] The durability of the humoral and cellular immune response after a single dose of an Ad26.RSV.FA2-vectored RSV vaccine was demonstrated for at least 6 months following vaccination and justifies this time window between vaccination and challenge. The 6-month immunogenicity data for Ad26.RSV.preF from study VAC18193RSV1003 will be available prior to vaccination and will be reviewed to further justify this strategy.

In the Unit, subjects will live in separate isolation rooms, and all personnel having contact with the subjects will wear isolation clothing, including personal protective equipment, to minimize the possibility of cross-contamination between subjects. On Day 0, each subject will receive the contents (0.8 mL) of 1 vial of virus inoculum.

From Day 2 to Day 11, nasal wash samples will be collected every 12 (±1) hours, abbreviated physical examination and vital signs measurements will be conducted daily, and SSCs will be filled out three times daily and reviewed by the attending physician. 24-hour tissue counts and mucus weights will be determined.

[a] The 7-day post-vaccination temperature measurements may be taken earlier to coincide with the corresponding clinic visit.

[b] Any overflow subject who is vaccinated but not challenged will be followed to the end of the study, or can be challenged at the discretion of the sponsor, providing study blind had not been broken. Immunogenicity blood draws for overflow subjects who are not challenged will occur at 28 days post-vaccination, and again 28 days after the date they would have originally been challenged on, to provide some vaccinated but not challenged controls for the study.

Nasopharyngeal samples will be taken on Day 12 and tested for the presence of virus prior to discharge using rapid virus antigen test (RVAT). Subjects will only be discharged on Day 12 if no detectable virus is present (negative RVAT). If symptoms are present, but no virus is detected (negative RVAT), discharge will be at the investigator's discretion. If appropriate, subjects may reside in quarantine for an additional night or longer before discharge. It is unlikely that subjects will transmit RSV virus to their close contacts after they have been released from the quarantine Unit. After infection with RSV, infectious virus will be present in the subject's nose for several days, but it is not expected to be present in the subject's nose at the time of discharge from the Unit. This is because the usual duration of time that RSV remains infective in adults is several days shorter than the time they will spend in the Unit in isolation.[9] Furthermore, at discharge, subjects will be negative by the RVAT assay.

To reduce any risk of passing RSV on to others, subjects will be advised to avoid contact with the following vulnerable groups of people for two weeks after they leave the Unit:

- any person with any known immunodeficiency
- any person receiving immunosuppressant medications
- any person undergoing or soon to undergo cancer chemotherapy
- any person who has congestive heart failure or is in frail health
- any person who has a diagnosis of emphysema or COPD, is elderly residing in a nursing home, or has severe lung disease
- any person who has received a transplant (bone marrow or solid organ)
- children below the age of 1 year
- elderly persons (aged 75 years or older)

Unsolicited AEs will be collected from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. SAEs will be collected from ICF signature until 6 months after vaccination. Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

Blood will be collected for sero-susceptibility (neutralizing antibody to RSV-A Memphis 37b) within 90 days of vaccination and on entry to the Unit. Blood and urine will be collected for laboratory safety assessments within 56 days of vaccination, pre-vaccination, at 7 days post-vaccination and prior to challenge on Day –1 or Day –2. Blood will additionally be collected for biochemistry and hematology assessments at discharge from the quarantine Unit and at 28 days post-challenge. Blood for assessment of cardiac enzymes will also collected on Days 3, 7, and 11.

Blood samples for humoral and cellular immunity will be collected pre-vaccination, 1-2 days prior to challenge, and at 28 days after challenge. *Note*: blood samples for immunogenicity

assessment will be collected from all subjects whether subjects are challenged or not (ie, including "overflow" subjects). Blood draws for overflow subjects who are not challenged will occur at 28 days post-vaccination and again 28 days later. Overflow subjects who are challenged in a later cohort will also have blood draws at the time of challenge and 28 days after challenge.

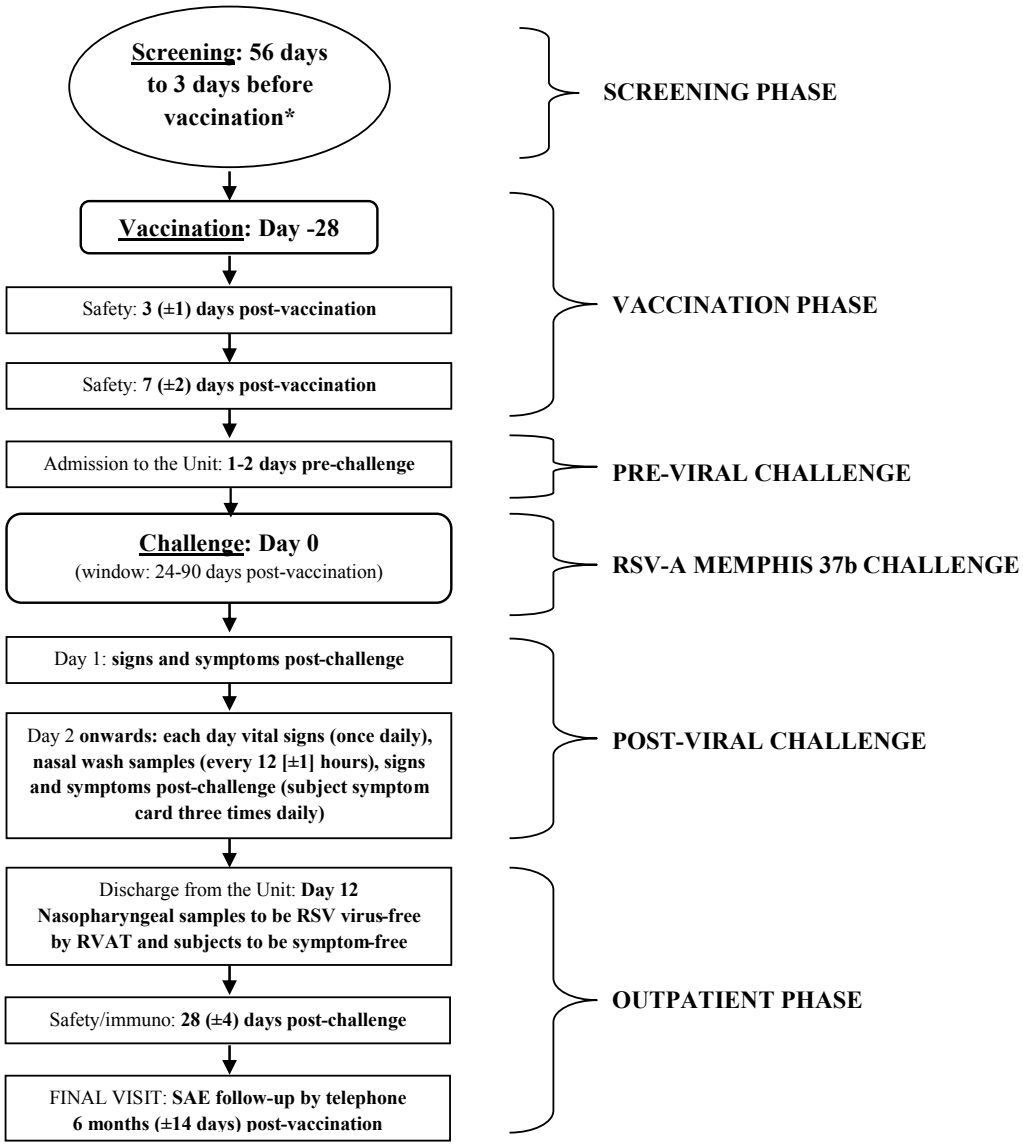
RSV pre-F antibody will be assessed on the day before challenge and at discharge from the Unit from nasal wash samples.

An internal data review committee (DRC) will be commissioned for this study, the members of which consist of a clinician with expertise in vaccines and infectious diseases, a medical safety officer and a statistician, none of whom will be part of the study team or involved in the conduct of the study. The DRC will meet if, in the judgment of the investigator and/or the sponsor's SRP, a significant or unexpected safety event occurs.

The end of the study will be the last subject's last visit by telephone at 6 months after vaccination.

A diagram of the study design is provided in Figure 1.

Figure 1: Schematic Overview of the Study



* Historical pre-screening data collected through the ethics committee-approved hVIVO screening protocol within 56 days to 3 days prior to vaccination (90 days for viral serology) may be used for screening procedures. Historical pre-screening data obtained prior to this window can be re-assessed any time from 40 days to 3 days prior to vaccination.
RVAT = rapid virus antigen test

3.2. Study Design Rationale

Dose Selection

The rationale behind selection of the Ad26 vector and dose selection is described in Section 1.1.

Availability of Safety Data Prior to Dosing

Two initial FIH studies (VAC18192RSV1001, N = 48; VAC18192RSV1003, N = 32) in healthy adults examining homologous and heterologous regimens of $5x10^{10}$ vp of Ad26.RSV.FA2 and

$5x10^{10}$ vp of Ad35.RSV.FA2 have been completed. Both Ad26.RSV.FA2 and Ad35.RSV.FA2 were shown to be safe and immunogenic.

One subsequent FIH study (VAC18193RSV1003, N = 72) in elderly adults in stable health examining homologous regimens of $5x10^{10}$ vp and $1x10^{11}$ vp of Ad26.RSV.preF is ongoing. All 72 subjects have been randomized and have received the first dose. Safety data from the unblinded interim analysis 28 days post-Dose 1 from all 72 subjects who received Ad26.RSV.preF ($5x10^{10}$ vp or $1x10^{11}$ vp) or placebo did not reveal any safety concerns.

4. SUBJECT POPULATION

The inclusion and exclusion criteria for enrolling subjects in this study are described in the following two subsections. If there is a question about the inclusion or exclusion criteria below, the investigator must consult with the appropriate sponsor representative and resolve any issues before enrolling a subject in the study. Waivers are not allowed.

Screening for eligible subjects will be performed between 56 days to 3 days before vaccination. Historical pre-screening data collected through the ethics committee-approved hVIVO screening protocol within 56 days (90 days for viral serology) to 3 days prior to vaccination may be used for screening procedures. Historical pre-screening data obtained prior to this window can be re-assessed any time from 40 days to 3 days prior to vaccination.

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

4.1. Inclusion Criteria

1 Each subject must sign an ICF indicating that he or she understands the purpose of and procedures required for the study, is willing to participate in the study and attend all scheduled visits, is willing to be isolated and stay in the clinic for the quarantine phase, and is willing and able to comply with all study procedures and adhere to the prohibitions and restrictions specified in this protocol.

2 Subject is a man or woman, ≥18 to ≤50 years old on the day of ICF signature.

3 Subjects must be in good health, without significant medical illness, on the basis of a medical evaluation that reveals the absence of any clinically relevant abnormality and includes a physical examination (including height and weight), skin examination, medical history, vital signs (systolic and diastolic blood pressure and heart rate, respiratory rate, and body temperature), and the results of clinical laboratory tests performed within 56 days of vaccination. If there are abnormalities, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

4 Subjects must have a non-clinically significant 12-lead ECG within 56 days of vaccination including:

a) normal sinus rhythm (heart rate between 50 and 100 beats per minute [bpm], extremes included)

b) QT interval corrected for heart rate according to Fridericia (QTcF) interval ≤450 ms

c) QT interval corrected for heart rate according to Bazett (QTcB) interval ≤450 ms

d) QRS interval <120 ms

e) PR interval ≤210 ms.

Note: Retesting of abnormal ECG values that may lead to exclusion will be allowed once without prior approval from the sponsor. Repeat ECG due to equipment failure will not count as a retest. Subjects with a normal value at retest may be included.

5 Subjects must be sero-suitable for RSV within 90 days of vaccination (low immunity to the RSV-A Memphis 37b virus using a virus neutralization assay).

6 Subject must be healthy on the basis of clinical laboratory tests performed within 56 days of vaccination. If the results of the laboratory screening tests are outside the local laboratory normal reference ranges and additionally within the limits of toxicity Grade 1 according to the US Food and Drug Administration (FDA) toxicity tables (ie, for tests in the FDA table[a]), the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant and appropriate and reasonable for the population under study. This determination must be recorded in the subject's source documents and initialed by the investigator.

Note: If laboratory screening tests are out of local laboratory normal ranges and deemed clinically significant, repeat of screening tests is permitted once using an unscheduled visit during the screening period to assess eligibility.

7 Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for subject participating in clinical studies.

Before randomization, a woman must be either:

a. Not of childbearing potential defined as:

i. Premenarchal: *a premenarchal state is one in which menarche has not yet occurred.*
ii. postmenopausal: *amenorrhea for at least 12 months without an alternative medical cause.*
iii. permanently sterile: *permanent sterilization methods include hysterectomy, bilateral salpingectomy, bilateral tubal occlusion/ligation procedures, and bilateral oophorectomy.*

b. Of childbearing potential and

[a] For the FDA toxicity grading tables, see Attachment 1: FDA Guidance document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007).

i. practicing an acceptable effective method of contraception. Acceptable methods for this study include:

- hormonal contraception;
- intrauterine device;
- intrauterine hormone-releasing system;
- male or female condom with or without spermicide;
- cap, diaphragm or sponge with a vaginal spermicide;
- vasectomized partner (the vasectomized partner should be the sole partner for that subject);
- sexual abstinence*.

Sexual abstinence is considered an effective method **only if defined as refraining from heterosexual intercourse from signing the informed consent until 4 months after study vaccination. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*

ii. agrees to remain on an effective method of contraception from signing the informed consent until 4 months after study vaccination. Use of hormonal contraception should start at least 28 days before the administration of study vaccine.

Note: If the childbearing potential changes after start of the study (eg, a premenarchal woman experiences menarche) or the risk of pregnancy changes (eg, a woman who is not heterosexually active becomes active), a woman must begin an acceptable effective method of contraception, as described above.

8 All female subjects must:

a. Have a negative highly sensitive serum β-human chorionic gonadotropin (β-hCG) pregnancy test within 56 days of vaccination

b. Have a negative urine β-hCG pregnancy test immediately prior to study vaccine administration

9 From the time of vaccination through 3 months after completion of the study, subject agrees not to donate blood.

4.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Subject has acute illness (this does not include minor illnesses such as diarrhea) or temperature ≥37.8 ºC within 24 hours prior to study vaccination.

2. Subject has a history of an underlying clinically significant acute or chronic medical condition or physical examination findings for which, in the opinion of the investigator, participation would not be in the best interest of the subject (eg, compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

3. Subject has history of malignancy (exceptions are basal cell carcinomas of the skin treated over 5 years prior to vaccination considered cured with minimal risk of recurrence).
4. Subject has had major surgery (per the investigator's judgment), within 12 weeks before dosing, or will not have fully recovered from surgery, or has surgery planned during the time the subject is expected to participate in the study or within 6 months after study vaccination. *Note*: Subjects with planned surgical procedures to be conducted under local or locoregional anesthesia and not judged as major by the investigator may participate.
5. Subject has chronic active hepatitis B or hepatitis C infection, documented by hepatitis B surface antigen and hepatitis C antibody, respectively.
6. Subjects with current human immunodeficiency virus type 1 (HIV-1) or HIV-2 infection.
7. Subject is in receipt of, or planning to receive, licensed live attenuated vaccine within 28 days of study vaccination and the viral challenge (ie, before and after); other licensed vaccines (ie, not live: eg, influenza, tetanus, hepatitis A, hepatitis B or rabies) should be given at least 14 days before or 14 days after study vaccination and the viral challenge.
8. Subject has received an investigational drug or used an invasive investigational medical device within 3 months or received an investigational vaccine within 6 months before the planned administration of study vaccine or is currently enrolled or plans to participate in another investigational study during the course of this study. *Note: Participation in an observational clinical study (ie, with no intervention) is allowed upon approval of the sponsor.*
9. Subject has previously participated in, or is currently participating in an RSV vaccine study.
10. Subject has previously received treatment with immunoglobulin. Subject has received treatment with blood products in the 4 months before the planned administration of study vaccine or has any plans to receive such treatment during the study.
11. Subject has a known allergy, or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine components (including any of the constituents of the study vaccine).
12. Criterion modified per Amendment 2:

    12.1 Subject has a history of chronic urticaria (recurrent hives), eczema and/or atopic dermatitis. *Note*: Subjects with a history of eczema only in childhood are allowed.
13. Subject has a history of acute polyneuropathy (eg, Guillain-Barré syndrome).
14. Subject has abnormal function of the immune system resulting from:

    - Clinical conditions (eg, autoimmune disease or immunodeficiency)
    - Chronic (longer than 10 days) or recurrent use of systemic corticosteroids during the study and within 6 months before administration of study vaccine (*Note*: ocular, topical or inhaled steroids are allowed)

- Administration of antineoplastic and immunomodulating agents or radiotherapy during the study and within 9 months before administration of study vaccine

15. Subject has a contraindication to intramuscular injections and blood draws, eg, bleeding disorders.
16. Subject is a woman who is pregnant, or breast-feeding, or planning to become pregnant while enrolled in this study or within 6 months after study vaccination.
17. Subject is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator, or an employee of the sponsor.
18. Subject cannot communicate reliably with the investigator.
19. Subject who, in the opinion of the investigator, is unlikely to adhere to the requirements of the study, or are unlikely to complete the full course of vaccination, challenge and observation.
20. Subjects with a past history of heart arrhythmias (extrasystoli, tachycardia at rest) or of risk factors for Torsade de Pointes syndrome (eg, hypokalemia, family history of long QT syndrome).
21. Subjects with a history or evidence of abuse of alcohol, barbiturates, amphetamines, recreational or narcotic drug use within 3 months prior to ICF signature, which in the investigator's opinion would compromise subject's safety and/or compliance with the study procedures.
22. Subjects with a positive urine drug test at screening. Urine will be tested for the presence of amphetamines, benzodiazepines, cocaine, cannabinoids, opioids, methadone, and barbiturates. *Note*: A positive urine drug test may be repeated once in the same sample, as soon as possible, to exclude a technical error. Subjects with a negative urine drug test at retest may be included.
23. Subject has a history of any illness that, in the opinion of the investigator, might confound the results of the study or that could prevent, limit or confound the protocol-specified assessments. This may include, but is not limited to, significant cardiac, vascular, pulmonary, gastrointestinal (such as significant diarrhea, gastric stasis, or constipation) that in the investigator's opinion could confound the challenge protocol assessments.

    Note: A history of childhood asthma before the age of 12 years is acceptable provided the subject is asymptomatic without treatment. Subjects with a single episode of wheezing after age 12 years (lasting less than eight weeks) can be included at the investigator's discretion provided the episode was more than four years ago and did not require a hospital admission and/or oral steroids.
24. Subjects having been previously experimentally inoculated with a virus from the same virus-family (Paramyxoviridae).

25. Subjects who have previously participated in another human viral challenge study with a respiratory virus in the preceding 12 months taken from the date of viral challenge in the previous study to the date of expected viral challenge in this study (subjects inoculated with influenza virus could participate in the current RSV study).
26. Vulnerable subjects (eg, incarcerated individuals).
27. Subjects with a lack of good/reasonable venous access.

Note: The investigator should ensure that all study enrollment criteria have been met prior to vaccination. If a subject's clinical status changes (including any available laboratory results or receipt of additional medical records) after ICF signature but before study vaccine is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study. Section 17.4, Source Documentation, describes the required documentation to support meeting the enrollment criteria.

4.3. Viral Challenge Exclusion Criteria

Any potential subject who meets any of the following criteria on entry to the quarantine Unit on Day –2 or –1 will be excluded from participating in the viral challenge.

1. Subject has acute illness within 7 days of admission (this does not include minor illnesses such as diarrhea). Subjects can be rescheduled for another viral challenge admission day if possible and the protocol window permitting.
2. Subject has a temperature ≥37.8 ºC on Day 0, the day of viral challenge. Subjects can be rescheduled for another viral challenge admission day if possible and the protocol window permitting.
3. Subjects having donated or lost more than 1 unit of blood (470 mL) within 60 days or more than one unit of plasma within 7 days.
4. Subjects with active acute respiratory infection.
5. Subject has any clinically significant abnormalities (ECG, vital signs).
6. Subjects having received an investigational agent (small molecule) or vaccine (other than study vaccine) within 30 days, or having received a biological product other than a vaccine within 3 months or 5 half-lives (whichever is longer) prior to entry to the quarantine Unit.
7. All female subjects must have a negative highly sensitive serum β-hCG pregnancy test on admission.
8. Subjects with a positive urine drug test. On admission, urine will be tested for the presence of amphetamines, benzodiazepines, cocaine, cannabinoids, opioids, methadone, and barbiturates. *Note*: A positive urine drug test may be repeated once in the same sample, as soon as possible, to exclude a technical error. Subjects with a negative urine drug test at retest may be included.

9. Male subjects must agree to the contraceptive requirements below at entry to the quarantine Unit, and continuing until 3 months after the date of viral challenge:

    - Use a condom and spermicide (for all partners)
    - Male sterilization with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate (note that the use of condom with spermicide will still be required to prevent partner exposure)
    - In addition, for female partners of childbearing potential, that partner must use another form of contraception such as:
        - Established (a minimum of 2 weeks prior to admission) use of oral, injected or implanted hormonal methods of contraception
        - Placement of an intrauterine device (IUD) or intrauterine system (IUS)
        - Barrier methods of contraception or occlusive cap (diaphragm or cervical/vault caps), both with one of the following: spermicidal foam/gel/film/cream/suppository
        - Bilateral tubal ligation
    - True abstinence – when this is in line with the preferred and usual lifestyle of the subject

    In addition to the contraceptive requirements above, male subjects must agree not to donate sperm following discharge from quarantine until 90 days after the date of viral challenge.

4.4. Prohibitions and Restrictions

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1 Agree to follow all requirements that must be met during the study as noted in the inclusion and exclusion criteria (Section 4.1 and Section 4.2, respectively).

2 Vaccination with live attenuated vaccines within 28 days of a study vaccination and the viral challenge (ie, before and after) is prohibited. Other vaccines (eg, influenza, tetanus, hepatitis A, hepatitis B, rabies) should be given at least 14 days before (or at least 14 days after) administration of study vaccine and the viral challenge in order to avoid potential confusion of adverse reactions and potential immune interference. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine.

3 Subjects may not use drugs such as amphetamines, benzodiazepines, cocaine, cannabinoids, barbiturates, opiates, and methadone from 3 months prior to ICF signature until completion of the last study-related activity. Refer to Section 8, for details regarding prohibited and restricted therapy during the study.

4 Subjects must not donate blood for 3 months after completion of the study.

5 Subjects must not consume any food containing poppy seeds or any codeine containing formulation starting 72 hours before screening and before admission to the quarantine Unit (in order to avoid false-positive urine drug screen).

6 Subjects must not smoke or otherwise use tobacco products from 30 days prior to entry to the quarantine Unit and must continue to abstain from smoking until the completion of the last study-related activity.

7 To reduce the risk of passing RSV on to others, subjects will be advised to avoid contact with the following vulnerable groups of people for two weeks after leaving the quarantine Unit:

- any person with any known immunodeficiency
- any person receiving immunosuppressant medications
- any person undergoing or soon to undergo cancer chemotherapy
- any person who has congestive heart failure or is in frail health
- any person who has a diagnosis of emphysema or COPD, is elderly residing in a nursing home, or has severe lung disease
- any person who has received a transplant (bone marrow or solid organ)
- children below the age of 1 year
- elderly persons (aged 75 years or older).

5. STUDY VACCINE ALLOCATION AND BLINDING

Study Vaccine Allocation

Within each cohort, subjects will be randomized 1:1 to one of the two groups (Ad26.RSV.preF or placebo). Randomizations will be based on computer-generated schedules prepared before the study by or under the supervision of the sponsor. The randomizations will be balanced by using randomly permuted blocks.

A unique code will dictate the study vaccine assignment for the subject.

Withdrawal of randomized subjects from vaccination before vaccination: Additional subjects will be recruited and immunized to replace these withdrawn subjects at the discretion of the sponsor. The replacement subject will be assigned to the same group as the original (discontinued) subject. The replacement subject's randomization number will equal the randomization number of the discontinued subject +100 (for example subject 0001 would be replaced by subject 0101).

As approximately 44 subjects need to be challenged, initially 4 more subjects in the first cohort will be randomized (overflow subjects). If subjects withdraw between vaccination and challenge, the unblinded pharmacist will make every effort to replace this subject with an overflow subject

of the same group as the withdrawn subject if possible. The withdrawal rate between vaccination and challenge for the first cohort will be used to assess how many extra subjects will need to be vaccinated for subsequent cohorts. Any subject who experiences an SAE before admission to the Unit will not be challenged and will be replaced.

Blinding

The investigator will be provided with a sealed randomization code for each subject, containing coded details of study vaccine allocation. All randomization codes, whether opened or sealed, will be collected after the end of the subject's participation in the study.

Under normal circumstances, the blind should not be broken until all subjects have completed the study and the database is finalized. While the responsibility to break the study vaccine allocation code in emergency situations resides solely with the investigator, it is recommended that the investigator contacts the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In an emergency situation, the investigator may determine the identity of the study vaccine by opening the sealed code. In the event the blind is broken, the sponsor must be informed as soon as possible. The date, time and reason for the unblinding must be documented in the appropriate section of the electronic case report form (eCRF). The investigator is advised not to reveal the study vaccine assignment to the study-site personnel or sponsor personnel. If the code is broken by the investigator or the study-site personnel, the subject must be withdrawn from the study and must be followed as appropriate. If the code is broken by the sponsor for safety reporting purposes, the subject may remain in the study.

The subjects, study-site personnel (including the vaccine administrator), and investigator will be blinded to study vaccine allocation throughout the study, except for the unblinded pharmacist or qualified staff member with primary responsibility for study vaccine preparation and dispensing.

At the end of the quarantine phase for the final cohort, the sponsor will request the treatment codes for the primary analysis. However, treatment codes will not be disclosed to the investigator and subject(s).

6. DOSAGE AND ADMINISTRATION

Ad26.RSV.preF will be supplied at a concentration of $1x10^{11}$ vp/0.5 mL in single-use vials, with an extractable volume of 0.5 mL, for intramuscular injection into the deltoid of the non-dominant arm. A dose level of $1x10^{11}$ vp will be used.

Placebo will be supplied as sterile 0.9% saline for injection.

For each subject, every vaccination will be 0.5 mL in volume.

An unblinded pharmacist, or other qualified individual will prepare the appropriate vial and syringe and provide the syringe in a blinded manner to the study vaccine administrator who will perform the injection.

Note: the unblinded pharmacist, or other qualified individual, may also perform the administration, but will have no other study function.

Further details on study vaccine preparation will be provided in the Investigational Product Preparation Instructions.

7. STUDY VACCINE COMPLIANCE

Study vaccine will be administered intramuscularly by a study vaccine administrator – a trained and qualified study nurse, medical doctor, or otherwise qualified health care professional. The date and time of study vaccine administration will be recorded in the eCRF.

8. PRE-STUDY AND CONCOMITANT THERAPY

Pre-study therapies administered up to 90 days before vaccination must be recorded in the eCRF at vaccination.

Concomitant therapies will be collected and recorded in the eCRF from time of study vaccine administration until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge, and additionally outside of these periods when associated with an SAE meeting the criteria outlined in Section 12.3.2. Information on concomitant use of herbal supplements or vitamins will not be collected.

Use of any experimental medication (including experimental vaccines other than the study vaccine) during the study is not allowed.

Subjects can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as needed, although their use must be documented and use of these medications as routine prophylaxis prior to study vaccination is discouraged, unless if specified by the sponsor. All other concomitant therapies should also be recorded if administered in conjunction with new or worsening AEs reported per protocol requirements outlined in Section 12.3.1).

Chronic (longer than 10 days) or recurrent use of systemic corticosteroids is prohibited during the study and within 6 months before administration of study vaccine (*Note*: ocular, topical or inhaled steroids are allowed). Antineoplastic and immunomodulating agents or radiotherapy are prohibited in the 9 months prior to vaccination and during the study.

After the viral challenge, paracetamol, oral contraception and thyroxin, if already prescribed before entry to the Unit, are allowed concomitant medications.

If chronic use of prohibited therapies becomes medically indicated during the course of the study for any subject, the sponsor should be contacted.

Vaccination with live attenuated vaccines within 28 days of a study vaccination and the viral challenge (ie, before and after) is prohibited. Other licensed vaccines (ie, not live) should be given at least 14 days before or at least 14 days after administration of study vaccine and the viral challenge in order to avoid potential confusion of adverse reactions and potential immune interference. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

9. STUDY EVALUATIONS

9.1. Study Procedures

9.1.1. Overview

Evaluation of the safety/tolerability of the vaccine regimen will include vital signs, physical assessment by study-site personnel, ECGs, clinical laboratory test data and subject reports on signs and symptoms following vaccinations. Additional visits may be required if, in the investigator's opinion, further clinical or laboratory evaluation is needed.

Each subject will be provided with a thermometer, ruler and subject diary to measure and record body temperature (tympanic or oral) and solicited local (at injection site) and systemic events. The diary includes instructions on how to capture the data and grading scales to assess severity of the symptoms. Study staff are responsible for providing appropriate training for diary completion to avoid missing or incorrect data. The subject diary will be reviewed by the study personnel at visits indicated in the Time and Events Schedule.

The Time and Events Schedule summarizes the frequency and timing of safety and immunogenicity measurements applicable to this study.

The total blood volume to be collected from each subject over the entire study will not exceed 470 mL unless additional blood samples are required for safety reasons. The maximum volume of blood to be drawn at any given visit will be 70 mL.

9.1.2. Visit Windows

For the following visits, windows will be allowed as indicated:

Visit Day	*Window*	*Primary Purpose*
Day –25	± 1 day	3-day post-vaccination, telephone call for safety
Day –21	± 2 days	7-day post-vaccination safety only visit
Challenge	24-90 days	Time between vaccination and viral challenge
28 days post-challenge	± 4 days	Safety and immunogenicity
6 months post-vaccination	± 14 days	Final visit (SAE follow-up)

9.1.3. Screening Phase

Screening for eligible subjects will be performed between 56 days to 3 days prior to vaccination. Historical pre-screening data from hVIVO's ethics committee-approved screening protocol can be used to confirm eligibility if performed within this time frame.

Only healthy subjects without acute illness or fever and complying with the inclusion and exclusion criteria specified in Section 4 will be included in the study. The investigator will provide detailed information on the study to the subjects and will obtain written informed consent prior to each subject's participation in the study.

The following evaluations will be performed to determine eligibility requirements as specified in the inclusion and exclusion criteria:

- Physical examination including vital signs measurement (respiratory rate, heart rate, supine systolic and diastolic blood pressure and body temperature) and height and weight
- Demographic information
- Medical history, including smoking habits
- Review of pre-study medications
- Review of inclusion/exclusion criteria
- 12-lead ECG
- Sero-susceptibility for RSV (neutralization assay)
- Serology testing (HIV type 1 or type 2, hepatitis B, hepatitis C)
- Blood sampling for hematology and biochemistry laboratory testing (laboratory tests at screening are to be done within 56 days of randomization)
- Urine sample for urinalysis
- Nasopharyngeal swab tolerability testing
- Nasal wash tolerability testing
- Spirometry
- All female subjects: serum β-hCG pregnancy testing
- A urine screen for drugs of abuse and an alcohol breath test
- Overall eligibility

Historical pre-screening data collected through the ethics committee-approved hVIVO screening protocol within 56 days (90 days for viral serology) to 3 days prior to vaccination may be used for screening procedures. Historical pre-screening data obtained prior to this window can be re-assessed any time from 40 days to 3 days prior to vaccination.

General eligibility for this clinical study will be dependent on results of laboratory tests and the medical assessment. Study subjects who qualify for inclusion based on the medical history, physical examination, and laboratory results will be contacted and scheduled for enrollment and vaccination. If necessary, the screening visit may be split into several visits. Screening should occur between 56 days to 3 days before vaccination.

Subjects with laboratory values not meeting eligibility criteria at the screening visit may have one repeat testing at the discretion of the investigator if the abnormality is not clinically significant and may be a testing aberrancy. Enrollment of a subject with laboratory values representing toxicity Grade 1 is allowed if the investigator considers the values not to be clinically significant and reasonable for the population under study. Details on toxicity grade assessment are provided in Section 12.1.3.

After laboratory data, medical history, physical examination, ECG and nasopharyngeal swab/nasal wash tolerance data have been reviewed for completeness and adherence to inclusion and exclusion criteria, the subject can be deemed eligible for the study.

Unsolicited AEs will be recorded on the AE page of the eCRF from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. SAEs will be collected from ICF signature until 6 months after vaccination (ie, the end of the study).

9.1.4. Vaccination Phase

9.1.4.1. Vaccination

Randomization/Vaccination

After re-check of inclusion and exclusion criteria[a], a urine screen for drugs of abuse, an alcohol breath test, abbreviated physical examination (at the discretion of the investigator) and measurement of vital signs, eligible subjects will be randomized as described in Section 5. If medical status and/or physical examination suggest significant changes have occurred since screening, the laboratory tests will be repeated and the vaccination visit rescheduled, or the subject excluded from the study if he/she fails to meet the inclusion and exclusion criteria. Pre-dose samples for baseline immunogenicity assessments and for clinical laboratory testing will be collected. All women must have a negative urine pregnancy test pre-dose. Before vaccination, the investigator must check for any symptoms of an acute illness or body temperature ≥37.8 °C. In such a situation, the subject may be re-screened (if vaccination number not assigned), or withdrawn at the discretion of the investigator. *Note*: the minimum time between vaccination and challenge is 24 days.

Administration of study vaccine. Vaccination will occur 28 days before challenge (window is not less than 24 or more than 90 days).

[a] To include exclusion criteria 1, 7, 8, 9, 10, 14 and 16.

Subjects will be closely observed for a minimum of 30 minutes post-vaccination to monitor for the development of any acute reactions, or longer if deemed necessary by the investigator. Any unsolicited, solicited local or systemic AEs and vital signs will be documented in the eCRF by study-site personnel following this observation period.

Subjects will be provided with a subject diary, thermometer, and ruler to measure and record body temperature, solicited local and systemic AEs for 7 days post-vaccination.

9.1.4.2. Post-vaccination Follow-Up

Sentinel Subjects: 48 Hours Post-first Vaccination

At 48 hours after the first vaccination, a telephone call will be made to the two sentinel subjects only to collect safety information (solicited and unsolicited AEs, SAEs and concomitant medications).

3 and 7 days post-vaccination

The visit at 3 days post-vaccination will be a telephone call to check subject diaries and to collect safety information (solicited and unsolicited AEs, SAEs and concomitant medications).

The visit at 7 days post-vaccination will include an abbreviated physical examination (at the discretion of the investigator), vital signs measurement, and recording of any AEs/SAEs and concomitant medications. Samples for clinical laboratory testing will be collected. Subject diaries will be reviewed and collected. If this visit occurs before the end of the post-vaccination diary period, review of the diaries will still take place, but diaries will be returned by subjects at the next visit (ie, on admission to the Unit for the challenge phase).

9.1.5. Viral Challenge Phase

9.1.5.1. Entry to the Quarantine Unit

Subjects will enter the quarantine Unit either one or two days before the viral challenge, to acclimatize and perform entry assessments. The following assessments can take place on either day: complete physical examination, including body weight, relevant medical information since vaccination, vital signs and spirometry, samples for clinical laboratory tests (including cardiac enzymes), ECG, blood samples for immunogenicity assessments, spirometry, and recording of any unsolicited AEs/SAEs and concomitant medications. Nasopharyngeal swabs and nasal wash samples will be collected to screen for respiratory virus and for RSV pre-F antibody assessment, respectively, and blood samples will be taken to determine sero-susceptibility for RSV for post-hoc reconfirmation (neutralization assay).

The SSC will be filled out by the subject and reviewed by the attending physician. Tissues for tissue counts and mucus weight will be distributed on Day –1. The first 24-hour collection will start on Day –1; tissue counts and mucus weights will be determined on Day 0.

A serum pregnancy test (β-hCG) will be performed for all female subjects. A urine drug screen, a urine cotinine test, and an alcohol breath test will be performed.

Throughout the challenge phase of the study from entry to the Unit until discharge, subjects will stay in individual isolation rooms to minimize cross-contamination between subjects.

Before challenge inoculation on Day 0, the subject's clinical status will be checked, including any available laboratory results or receipt of additional medical records.

9.1.5.2. Challenge

On Day 0, intranasal challenge with RSV-A Memphis 37b virus will occur for all subjects. The site physician, or inoculation-trained member of the site medical team, will inoculate each subject intranasally with 0.8 mL of virus, delivered by pipetting 2x200 µL of virus per nostril. The target day for challenge is 28 days after vaccination (window is not less than 24 or more than 90 days). The longer window will be for the few overflow subjects that are available for challenge in a subsequent cohort.

Additionally, all post-challenge procedures per Section 9.1.5.3 will be carried out apart from collection of a nasal wash sample and ECG.

The inoculum virus titer is determined in an infectivity (plaque) assay, the titer is reported in plaque forming units per mL (PFU/mL). The challenge dose is approximately $4\log_{10}$ PFU. One vial of challenge virus will be provided for each subject. Full details of inoculation procedures will be provided in the Analytical Plan.

9.1.5.3. Post-challenge

On each of the 11 days after the challenge, procedures will include a directed physical examination, spirometry and monitoring of vital signs. Nasal wash samples will be taken every 12 (±1) hours from the second day after challenge until discharge. The SSC will be filled out three times a day by the subject and reviewed by the attending physician. Tissue counts and mucus weight will be determined for each 24-hour interval. ECGs will be carried out, and blood samples collected for clinical laboratory tests (cardiac enzymes only) on Days 3, 7 and 11. Any unsolicited AEs/SAEs and concomitant medications will be recorded.

9.1.5.4. Discharge from the Unit

On Day 12, subjects will only be discharged when no detectable virus is present (negative RVAT). If symptoms are present, but no virus is detected (negative RVAT), discharge will be at the investigator's discretion. If appropriate, subjects may reside in quarantine for an additional night or longer before discharge.

Procedures will include a complete physical examination including body weight, vital signs and spirometry, collection of nasopharyngeal swabs, and completion of the SSC by the subject and review by the attending physician. Tissue counts and mucus weight will be determined for the final 24-hour interval, and nasal wash samples will be collected for RSV pre-F antibody

assessment. A urine pregnancy test will be performed for all female subjects. Any unsolicited AEs/SAEs and concomitant medications will be recorded, and blood samples collected for clinical laboratory tests (hematology and biochemistry only).

9.1.6. Day 28 Post-challenge

The visit at 28 days post-challenge will include an abbreviated physical examination (at the discretion of the investigator), and vital signs measurement. Blood samples for immunogenicity assessments will be collected. Any unsolicited AEs/SAEs and concomitant medications will be recorded, and blood samples collected for clinical laboratory tests (hematology and biochemistry only). A urine pregnancy test will be performed for all female subjects.

9.1.7. Final Visit

The final visit at 6 months post-challenge will be a telephone call to record SAEs and concomitant medications associated with any SAE.

9.1.8. Early Withdrawal – Early Exit Visit

For those subjects who are unable to continue participation in the study, but who do not withdraw consent, an early exit visit will be conducted as soon as possible. In the event of early withdrawal from the study, all procedures required at the Day 28 visit (Section 9.1.6) will be performed. Samples for immunogenicity assessments will only be collected if the early exit is at least 14 days after the previous blood draw for immunogenicity. Samples for safety laboratory testing will only be collected if the early exit is within 7 days of the vaccination. Solicited AEs will be recorded if the early exit visit occurs within 7 days of vaccination. Any unsolicited AEs/SAEs and concomitant medications will be recorded. At the early exit visit, a serum pregnancy test (β-hCG), not a urine test, will be performed for all female subjects.

Precautions for any subject leaving the challenge Unit are listed in Section 4.4.

9.1.9. Overflow Subjects

The timing of immunogenicity blood draws for overflow subjects will be as follows:

- Any overflow subject who is challenged in a later cohort: blood draws at the time of challenge and 28 days after challenge.
- Any overflow subject who is not challenged: blood draws at 28 days post-vaccination, and again 28 days after the date they would have originally been challenged on.

Any overflow subject who is vaccinated but not challenged will be followed to the end of the study. Unsolicited AEs will be collected until 28 days after vaccination; SAEs will be collected until the end of the study.

Alternatively, these subjects can be challenged at the discretion of the sponsor, providing the study blind has not been broken.

9.2. Study Evaluations

9.2.1. Immunogenicity

Venous blood samples of approximately 10 mL and 50 mL will be collected for the determination of humoral and cellular responses, respectively, pre-vaccination, one or two days before viral challenge, and at 28 days post-challenge, or at the early exit visit if the subject prematurely terminates the study, without withdrawing consent.

All sample collection and processing will be performed by the staff at the clinical site according to current versions of approved standard operating procedures.

Humoral and cellular immunogenicity evaluations are summarized in Table 5 and Table 6, respectively.

Table 5: Summary of Immunogenicity Assays (Humoral)

Assay	Purpose
Exploratory endpoints	
RSV neutralization RSV-A Memphis 37b	Measure levels of the antibody to the challenge strain
Intranasal pre-F and post-F antibody*	Measure levels of vaccine induced antibody at the site of infection
RSV neutralization A2	Analysis of neutralizing antibodies to the A2 strain
RSV strain cross-neutralization	Analysis of cross-neutralizing antibodies to B and/or a different A strain
F-protein antibody (ELISA; pre- and/or post-fusion)	Analysis of antibodies binding to RSV F protein in post-fusion and/or pre-fusion form
RSV G/N ELISA	Analysis of binding antibodies to G and N proteins of RSV
Adenovirus neutralization assay	Analysis of neutralizing antibodies to adenovirus
Functional and molecular antibody characterization	Analysis of antibody characteristics including but not limited to ADCC, ADCP, avidity, Fc characteristics, Ig isotype

*From nasal wash samples, if the ELISA will be available
ADCC = antibody-dependent cell-mediated cytotoxicity; ADCP = antibody-dependent cellular phagocytosis; ELISA = enzyme-linked immunosorbent assay; F = fusion; Ig = immunoglobulin; RSV = respiratory syncytial virus

Table 6: Summary of Immunogenicity Assays (Cellular)

Assay	Purpose
Exploratory endpoints	
IFNγ ELISpot	T-cell IFNγ responses to RSV F-protein peptides
Flow cytometry (ICS)	Analysis of T-cell responses to RSV F-protein peptides (including, but not limited to, CD4/CD8, IL2, IFNγ, TNFα and/or activation markers, memory, Th1/Th2 subtyping)
Cytokine analysis	Analysis of secreted cytokines in RSV F peptide-stimulated PBMC supernatant, including, but not limited to, measurement of Th1/Th2 cytokine balance

ELISpot = enzyme-linked immunospot; F = fusion; ICS = intracellular cytokine staining; IFNγ = interferon gamma; IL = interleukin; PBMC = peripheral blood mononuclear cell; Th = T-helper (cell); RSV = respiratory syncytial virus; TNFα = tumor necrosis factor alpha

In addition to RT-PCR performed on nasal wash samples collected from Day 2 following challenge, any immunogenicity blood sample collected from a subject who subsequently undergoes challenge may also be assayed by a serological assay (eg, ELISA specific to RSV

protein G and/or N) to confirm RSV infection and evaluate the relationship between these assays and virological and clinical findings.

Nasal wash samples collected pre- and post-challenge and at discharge from the Unit will be used to assess any immunoglobulin or cellular immune component and its possible relation to viral load and clinical findings.

Instructions for the collection, handling, storage, and shipment of blood and nasal wash samples for immunogenicity assay are found in the Laboratory Manual that will be provided. Collection, handling, storage, and shipment of blood and nasal wash samples to the central laboratory must be under the specified, and where applicable, controlled temperature conditions as indicated in the Laboratory Manual.

9.2.2. Antiviral Activity Samples for Infectivity

Respiratory syncytial virus viral load will be measured in nasal washes (in both nostrils), using quantitative RT-PCR and a cell-based infectivity assay. Samples for the determination of RSV levels will be taken at several timepoints during the study, as indicated in the Time and Events Schedule. The procedures for sample processing and storage will be provided in the hVIVO Analytical Plan. The changes in RSV viral load will not be reported as AEs or SAEs.

- RSV neutralization assay

  To be eligible for the study a subject must be ‘sero-suitable’. Each subject will be initially screened for eligibility based on low immunity to the RSV-A Memphis 37b virus using a virus neutralization assay. This assay will be performed within 90 days of vaccination and on admission to the Unit (for post-hoc confirmation of RSV sero-susceptibility).

- Cell-based infectivity assay

  Details will be provided in the Laboratory Manual.

- PCR for viral load: quantitative RT-PCR

  The viral ribonucleic acid (RNA) will be extracted from the nasal wash, complementary deoxyribonucleic acid (cDNA) will be generated and quantitative RT-PCR of the N gene of the RSV strain will be performed. The quantitative RT-PCR will inform on the presence or absence of the RSV in the nasal wash and if the RSV is present the quantitative RT-PCR will provide the amount of virus (plaque forming unit equivalents [PFUe] per mL).

9.2.3. Mucus Weight and Tissue Count

At several timepoints during the study, as is indicated in the Time and Events Schedule, the mucus weight (nasal) will be assessed.

Subjects will be given pre-weighed packets of paper tissues (handkerchiefs). After each tissue is used for nose blowing or sneezing, the subjects should store them in an airtight plastic bag.

Paper tissue and bag distribution first takes place on Study Day –1 at 08:00 a.m. (± 30 minutes). Paper tissue collection starts from Study Day 1 through the day of discharge at approximately the same time.

Subjects will be instructed to place used single tissues into a specified bag which will be collected the following morning. A daily 24-hour collection will take place and new bags will be distributed.

All paper tissues used by each subject will be collected for each 24-hour period throughout the quarantine phase, to determine daily mucus weight and number of tissues used.

Used and unused tissues will be counted. In case of missing tissues, they will be replaced by new dry tissues, and the subjects will be re-educated about proper tissue use and collection.

Collection time of the plastic bags, daily mucus weight, and number of tissues used should be recorded on the eCRF.

Tissues will not be stored after the mucus weight is determined.

9.2.4. RSV Subject Symptom Card

At several timepoints during the study, as indicated in the Time and Events Schedule, all subjects will complete the SSC in order to assess the clinical symptoms of acute RSV infection (see Attachment 3).[8] For each timepoint the results will be transcribed onto the eCRF by a member of the study-site personnel, and will be summarized according to the instructions.

The duration and severity of self-reported symptoms, clinical symptoms, respiratory tract illness, and systemic illnesses will be assessed and compared between treatment groups.

The investigator will review the subject's SSC entries on a daily basis after the administration of challenge virus. Subject symptom scores (self-assessment form), including body temperatures, and AEs will be conducted and daily during quarantine.

From challenge until discharge from the Unit, SSC findings greater than Grade 0 will be presumed to represent virus infection consequent to challenge, and will not additionally be captured as an AE, unless they meet the criteria for an SAE. The changes in RSV viral load will not be reported as AEs or SAEs.

9.2.5. Respiratory Parameters: Spirometry

Spirometry (FEV_1, FVC, FEV_1/FVC) will be performed at several timepoints as specified in the Time and Events Schedule. Spirometry may also be investigated at any time following respiratory signs or symptoms (repeated coughing, bradypnea, tachypnea, rales and rhonchi) of respiratory difficulties. Spirometry will be performed as per hVIVO's Operating Instruction. In case of respiratory difficulties, the investigator may decide to perform additional lung function testing.

Spirometry will be done for each subject at approximately the same timepoint on each day from Day 0.

At each timepoint, three technically acceptable measurements will be recorded using a spirometer. The best reading from each assessment (as provided by the spirometry machine) will be used for analysis.

The following data will be automatically calculated:

- FEV_1 % predicted;
- FEV_1/FVC ratio (absolute, not predicted);

Any clinically relevant changes in pulmonary function occurring during the study will be recorded on the Adverse Event Section of the eCRF.

9.2.6. Safety Evaluations

Any clinically relevant changes occurring from ICF signature until discharge from the Unit must be recorded on the eCRF. Any clinically significant abnormalities, including those persisting at the end of the study/early withdrawal, will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

Adverse events occurring form ICF signature until 28 days after vaccination will be examined for relationship to study vaccine. Adverse events occurring from admission to the quarantine Unit until 28 days after challenge will be evaluated for relationship to the challenge.

The study will include the evaluations of safety and tolerability outlined in the following sections according to the timepoints provided in the Time and Events Schedule.

9.2.6.1. Adverse Events

Unsolicited AEs will be reported by the subject from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. Solicited AEs will be reported by the subject for 7 days after vaccination via the subject diary. Adverse events will be followed by the investigator as specified in Section 12.3.

For solicited AEs, the following applies:

- **Solicited Adverse Events**

Information related to solicited events as defined in Section 12.1.1, will be recorded by subjects in a diary for 7 days after vaccination. Each subject will be provided with a diary and instructions on how to complete the diary (Section 9.1.1). There will be a minimum 30-minute post-vaccination assessment of solicited events at the site. Diary information will be transcribed by the study personnel in the appropriate eCRF pages.

Subjects will be asked to note in the diary occurrences of pain/tenderness, erythema and induration/swelling at the study vaccine injection site daily for 7 days post-vaccination. The extent (largest diameter) of any erythema, and induration/swelling should be measured (using the ruler supplied) and recorded daily. Induration/swelling should also be graded using the functional scale.

- **Injection Site Pain/Tenderness**

  Injection site pain (eg, stinging, burning) is an unpleasant sensory and emotional experience associated with actual or potential tissue damage and occurring at the immunization site (with or without involvement of surrounding tissue). Injection site tenderness is a painful sensation localized at the injection site upon palpation and/or movement of the limb. Due to subjective nature of the reaction, the severity assessment of pain/tenderness is self-reported (if a subject is unable to provide self-report, other reporters include family member/caregiver or health care provider).[12]

- **Injection Site Erythema**

  Injection site erythema is a redness of the skin caused by dilatation and congestion of the capillaries localized at the injection site. It can best be described by looking and measuring.

- **Injection Site Swelling/Induration**

  Injection site swelling is a visible enlargement of an injected limb. It may be either soft (typically) or firm (less typical). Injection site induration is a palpable thickening, firmness, or hardening of soft tissue, usually has well-demarcated palpable borders, can be visible (raised or sunken compared to surrounding skin), is often 'woody' to touch and has a flat shape. As differentiation between swelling and induration may be difficult without health care professional's assessment, both symptoms have been combined to allow self-assessment by the subjects. Both swelling and induration can best be described by looking and measuring.

<u>*Note*</u>: any other injection site events not meeting the above case definitions should be reported separately as unsolicited AEs.[20,21]

Systemic Adverse Events

Subjects will be instructed on how to record daily temperature using a thermometer provided for home use. Subjects should record body temperatures in the diary in the evening of the day of vaccination, and then daily for the next 7 days approximately at the same time each day.[a] If more than one measurement is made on any given day, the highest temperature of that day will be used in the eCRF.

[a] The 7-day post-vaccination temperature measurements may be taken earlier to coincide with the corresponding clinic visit.

Fever is defined as endogenous elevation of body temperature ≥38° C, as recorded in at least one measurement.[24]

Subjects will also be instructed on how to note daily in the diary for 7 days after vaccination symptoms of the following events: fatigue, headache, myalgia, arthralgia, chills, nausea, and fever (ie, body temperature ≥38 °C).

The severity of these solicited systemic AEs will be graded by the investigator according to the criteria presented in Section 12.1.3.

If a solicited local or systemic AE is not resolved by 7 days after vaccination, the follow-up will be captured on the diary. The subject will be instructed to record the date of last symptoms and maximum severity in the diary after resolution.

9.2.6.2. Clinical Laboratory Tests

Blood and urine samples for laboratory safety assessments will be collected for screening up to 56 days prior to vaccination, pre-vaccination, 7 days post-vaccination, and prior to challenge on Day –1 or Day –2. Blood samples will additionally be collected at discharge from the quarantine Unit and at 28 days post-challenge. The following tests will be performed by a local laboratory (*parameters only measured at screening):

- **Hematology Panel**

  platelet count.
  white blood cell (WBC) count (absolute)
  WBC differential
  neutrophils
  lymphocyte
  monocytes
  eosinophils
  basophils
  red blood cell (RBC) count
  reticulocyte count (% and absolute)
  hemoglobin
  hematocrit
  mean corpuscular volume (MCV)
  mean corpuscular hemoglobin (MCH)
  MCH concentration (MCHC)

- **Biochemistry Panel**

  sodium
  potassium
  glucose (random)
  albumin

chloride
bicarbonate
calcium
uric acid
total protein
creatinine
total, direct, and indirect bilirubin
inorganic phosphate
blood urea nitrogen
C-reactive protein
gamma glutamyl transferase
alkaline phosphatase
alanine transaminase
lactate dehydrogenase
aspartate transaminase
urea

- **Cardiac Enzymes** (done together with ECGs on admission, and on Days 3, 7 and 11)

  Troponin T
  creatinine kinase

- **Urinalysis** – dipstick for:

  specific gravity
  pH
  glucose
  protein
  blood
  ketones
  appearance
  color
  nitrite
  urobilinogen
  bilirubin
  leucocytes

A repeat urinalysis will be performed in the event of a positive dipstick test. Microscopic reflex testing will be carried out if the repeat urinalysis dipstick test is also positive.

Review and Grading of Laboratory Data

The investigator must review each laboratory result, document this review and assess systematically any clinical significance. The laboratory reports must be filed with the source documents.

Laboratory values will be initially evaluated by the investigator according to local laboratory criteria. Abnormal values outside the local laboratory range of normal will be graded according to the FDA Guidance document "Toxicity Grading Scale from Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (see Attachment 1). Laboratory values within local laboratory normal limits will not be FDA graded and will be considered as normal.

Reporting Laboratory Abnormalities as AEs

Any clinically significant abnormal laboratory value within 28 days post-vaccination that falls outside of the local laboratory normal range and that requires follow-up will be captured as an AE. Laboratory values outside normal ranges that are not clinically significant in the judgment of the investigator, should not be recorded as AEs.

Any laboratory value falling within the local laboratory normal range will not be severity graded or recorded as an AE, regardless of whether the value falls within FDA ranges for Grade 1 or higher.

Note: Values for parameters falling within the local laboratory normal range should not be reported as AEs.

Repeat of Clinically Significant Laboratory Tests

For any clinically significant abnormal laboratory value that has increased in grade over baseline, the test must be repeated at the next scheduled visit or sooner based on the investigator's judgment, however Grade 3 abnormalities should be retested within 48 hours. Any clinically significant abnormalities (including those persisting at the end of the study or at early withdrawal) will be followed by the investigator until resolution or until a clinically stable endpoint is reached (see Section 12.3).

Screening Procedures

For entry into the study, each subject must be healthy on the basis of clinical laboratory tests performed within 56 days of vaccination. Enrollment of a subject with laboratory values outside of the local laboratory normal range representing FDA toxicity Grade 1 is allowed if the investigator considers the values reasonable for the population under study and not clinically significant.

Additional Clinical Laboratory Assessments

Additional clinical laboratory assessments to be performed are as follows:

- Serology testing (HIV type 1 or type 2, hepatitis B, hepatitis C) within 56 days of vaccination
- Serum pregnancy testing (β-hCG) for all female subjects within 56 days of vaccination and at entry to the quarantine Unit

- Urine pregnancy testing for all female subjects pre-vaccination
- Urine drug screen at screening and at entry to the quarantine Unit

9.2.6.3. Electrocardiogram

Supine 12-lead ECGs will be performed within 56 days of vaccination and on Day –1 or Day –2 prior to challenge; ECGs will only be performed at other times during the study if clinically indicated based on signs and symptoms. ECGs will be interpreted locally.

For 30 minutes prior to the ECG, subjects should refrain from meals, hot or cold beverages and strenuous exercise, and should remain in a room with a comfortable temperature. Each ECG should be obtained after the subject has been at rest for at least 5 minutes.

Enrollment of a subject with abnormal ECG results is allowed as long as the investigator feels that these are not clinically significant and appropriate for the population.

9.2.6.4. Vital Signs

Supine blood pressure and pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (eg, television, cell phones).

The following measurements will be performed:

- Heart rate (beats per minutes, bpm), respiratory rate (breaths per minute), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg)
- Body temperature (tympanic or oral route)

Confirmatory vital signs measurement can be performed if inconsistent with a prior measurement. If any clinically significant changes in vital signs are noted, they will be reported as AEs and followed to resolution, or until reaching a clinically stable endpoint.

9.2.6.5. Physical Examination

A full physical examination will be carried out within 56 days of vaccination, on admission to the Unit, and on discharge from the Unit. At all other visits, an abbreviated, symptom-directed examination will be performed by the investigator based on any clinically relevant issues, clinically relevant symptoms and medical history. Symptom-directed physical examination may be repeated if deemed necessary by the investigator.

Physical examinations will be performed by the investigator or appropriately trained delegate. Any abnormalities or changes in severity noted during the review of body systems should be documented in the eCRF.

10. SUBJECT COMPLETION/DISCONTINUATION OF STUDY VACCINE/ WITHDRAWAL FROM THE STUDY

10.1. Completion

A subject will be considered to have completed the study challenge if he or she has been challenged in the quarantine Unit and remained until meeting the requirements for discharge from the Unit. A subject will be considered to have completed the study if he or she has completed assessments at the final visit at 6 months post-vaccination.

10.2. Discontinuation from Challenge Phase/Withdrawal from the Study

Discontinuation from Challenge Phase

Subjects will be discontinued prior to the viral challenge phase (ie, pre-inoculation) for the reasons listed below. These subjects must not receive the viral challenge but should continue other study procedures, eg, safety follow-up:

- Pregnancy
- Any SAE
- Any related AE, worsening of health status or intercurrent illness that, in the opinion of the investigator, precludes the subject from viral challenge
- The randomization code is broken by the investigator or the study-site personnel

Overflow subjects from the first cohort will be challenged in subsequent cohorts. Any subject who is vaccinated in subsequent cohorts but not challenged will be followed to the end of the study.

Withdrawal from the Study

Each subject has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator. Although the subject is not obliged to give a reason for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the subject's rights.

A subject will be withdrawn from the study for any of the following reasons:

- Repeated failure to comply with protocol requirements
- Decision by the sponsor or the investigator to stop or cancel the study
- Decision by local regulatory authorities and Institutional Review Board/Independent Ethics Committee (IRB/IEC) to stop or cancel the study
- Lost to follow-up
- Withdrawal of consent

- Death

Any unnecessary study discontinuation should be avoided. Should a subject be withdrawn, all efforts should be made to complete and report the observations as thoroughly as possible. Whenever a subject is withdrawn from the study, independent of the reason, a final evaluation should be completed for that subject and the major reason for which the subject was withdrawn must be stated. If a subject is lost to follow-up, every reasonable effort must be made by the study-site personnel to contact the subject and determine the reason for withdrawal. The measures taken to follow-up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the eCRF and in the source document. Study vaccine assigned to the withdrawn subject may not be assigned to another subject. In general, subjects who withdraw will not be replaced, unless that subject was randomized but did not receive any study vaccine. Any subject who withdraws from the study after vaccination but before challenge will be replaced in the challenge by an overflow subject.

For those subjects who are unable to continue participation in the study, but for whom consent is not withdrawn, an exit visit will be conducted as soon as possible (see Section 9.1.8).

Subjects who wish to withdraw consent from participation in the study will be offered a single exit visit for safety follow-up (prior to formal withdrawal of consent). They have the right to refuse.

Precautions for any subject leaving the challenge Unit are listed in Section 4.3.

Withdrawal from the Use of Samples in Future Research

The subject may withdraw consent for use of samples for future research (refer to Section 16.2.5). In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of the sample retention for research are presented in the main ICF.

10.3. Contraindications to Vaccination

The following events constitute a contraindication to vaccination at that point in time. If any of these events occur at the scheduled time for vaccination, the subject may be re-screened (if vaccination number not assigned), or withdrawn at the discretion of the investigator and after consultation with the sponsor. *Note*: the minimum time between vaccination and challenge is 24 days:

- Severe acute illness at the time of vaccination. This does not include minor illnesses such as diarrhea.
- Fever (body temperature ≥37.8 °C) at the time of vaccination.

Note: medically indicated vaccines should be given at least 14 days before or 14 days after study vaccine administration and the viral challenge (see Section 4.3).

11. STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the immunogenicity and safety data is outlined below. Specific details will be provided in the SAP.

Planned analyses are described in Section 11.8.

11.1. Analysis Sets

The **Full Analysis (FA) Set** includes all subjects who were randomized and received study vaccine, regardless of the occurrence of protocol deviations. Vaccination assignment will follow the as-treated principle. All safety and subject information analyses will be based on the FA set. As a sensitivity analysis, key immunogenicity tables will also be based on the FA set.

The **Intent-to-Treat-Challenge (ITTc)** population is a subset of the FA set that includes all randomized, vaccinated and challenged subjects. All efficacy analyses will be performed on the ITTc population. Important subject information tables might be repeated based on the ITTc population as well.

The **Per-protocol Immunogenicity (PPI) Set** will include all randomized and vaccinated subjects for whom immunogenicity data are available, excluding subjects with major protocol deviations expecting to impact the immunogenicity outcomes.

In addition, for subjects who experience a natural RSV infection (based on RT-PCR, or other sources), samples taken after the natural infection will not be included in the PPI set.

The analysis of immunogenicity will be based on the PPI set.

11.2. Sample Size Determination

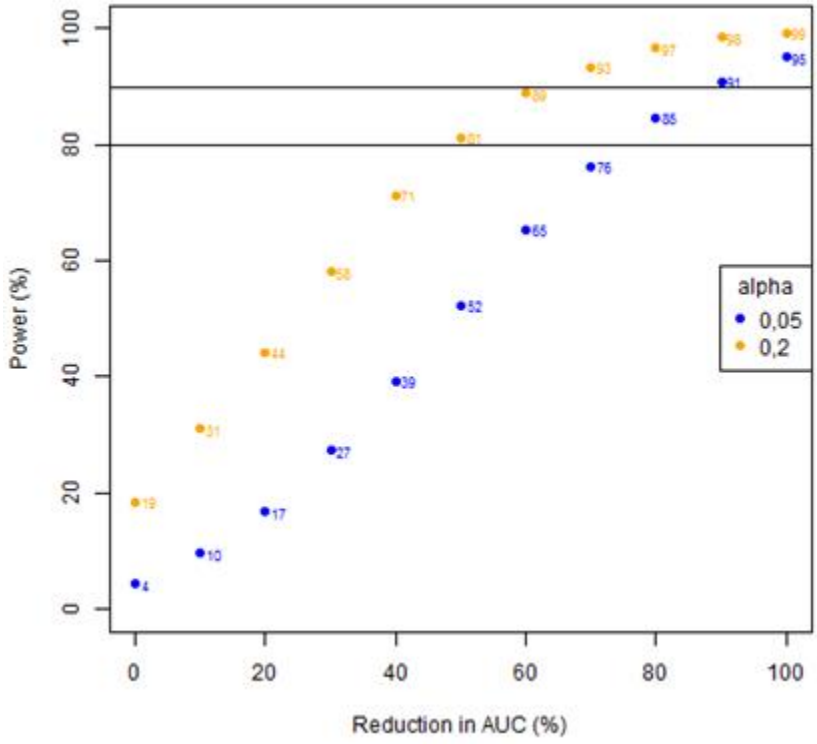
A sample size of 44 challenged subjects (22 vaccinated and 22 placebo subjects) was chosen as this would provide sufficient evidence to assess if the challenge model is a reasonable approach to examine vaccine efficacy. A multiple of 22 subjects was selected given the capacity of the quarantine Unit.

The sample size was based on the ability to detect trends with a high one-sided alpha (20%), given this is an exploratory study whose partial aim is to determine the utility of the RSV human challenge model for RSV vaccine development. Figure 2 shows the expected power for several reductions in VL-AUC of the quantitative RT-PCR assay, with this selected sample size, a mean VL-AUC in the placebo group of 320 $\log_{10}$ h/mL with corresponding standard deviation 275, an infection rate in placebo of 65% and a one-sided alpha of 5% and 20% based on a Wilcoxon Rank Sum test. This graph suggests that for a one-sided alpha level of 20%, the study has at least 80% power if Ad26.RSV.preF induces a reduction of VL-AUC of the quantitative RT-PCR assay by 50% or more. For a one-sided alpha level of 5%, the study has at least 80% power if Ad26.RSV.preF induces a reduction of VL-AUC of the quantitative RT-PCR assay by 75% or more.

An effect that is significant at 5% (one-sided) will be considered a significant effect. An effect that is significant at 20% (one-sided) will be considered as a trend. This trend, if observed, might be confirmed in future studies.

As this is an exploratory study, the statistical assumptions described above will need to be demonstrated to be correct for the analysis to be conducted as indicated below and as planned in further detail in the SAP. For example, if the infection rate from the study is considerably below 65% then the study will be considered to be underpowered to be able to accurately determine the stated aims. The infection rate in the placebo group will be followed on an ongoing basis by an unblinded individual who does not have any other study function to ensure a sufficient infection rate has occurred. If the infection rate is lower than anticipated, additional subjects may be enrolled, initially four additional subjects in each arm and up to 70 in total. This is to ensure that a high enough number of evaluable subjects is reached in each arm.

Figure 2: Expected Power for Several Reductions in Viral Load



11.3. Subject Information

For all subjects, demographic characteristics (eg, age, height, weight, body mass index [BMI], race, and gender), and other baseline characteristics (eg, physical examination, medical history, and concomitant diseases) will be tabulated and summarized with descriptive statistics.

11.4. Efficacy Analyses

For efficacy, baseline is defined as the last assessment challenge with the virus.

The **primary efficacy endpoint** is the VL-AUC of RSV-A Memphis 37b as determined by quantitative RT-PCR assay of nasal wash samples. The VL-AUC is calculated based on the viral load values measured twice daily, starting with the baseline value, and ending with the last available value before discharge.

For this primary efficacy endpoint the exact Wilcoxon Rank Sum test will be performed and the one-sided p-value will be interpreted at the 5% and 20% significance level, specifically only analysing the statistical significance of a reduction in VL-AUC in the active versus placebo groups.

The **secondary efficacy endpoints** are:

- The peak viral load of RSV-A Memphis 37b, defined as the maximum viral load as determined by quantitative RT-PCR assay of nasal wash samples, observed over the entire time period
- The effect of vaccination on viral load and symptoms on Day 6 and Day 7
- The viral load of RSV-A Memphis 37b as determined by quantitative RT-PCR assay of nasal wash samples over time
- The VL-AUC of RSV-A Memphis 37b as determined by quantitative culture of RSV of nasal wash samples and the corresponding AUC
- Weight of mucus produced over time and the corresponding AUC, tissue count
- Proportion of subjects with symptomatic RSV infection. Symptomatic RSV infection is defined in two ways:
    - Conservative: the subject has two or more quantifiable RT-PCR measurements on different samples **and** the subject has one of the following
        - symptoms from two different categories (Upper Respiratory, Lower Respiratory, Systemic) from the SSC, regardless of grade and assessment timepoint.
        - any Grade 2 symptom from any category.
    - Liberal: two or more quantifiable RT-PCR measurements plus any clinical symptom of any severity.

    Note: the quantifiable RT-PCR assessment and the symptoms should not necessarily occur at the same day.

Exploratory efficacy endpoints are:

- Proportion of subjects with symptomatic RSV infection (RT-PCR or serology). Symptomatic RSV infection is defined as having two or more quantifiable RT-PCR measurements or serology confirmation of RSV, and any clinical symptom of any severity.
- Total clinical symptom score (using a composite of 10 and 13 self-reported symptoms on the SSC) over time and the corresponding AUC.

- To determine the most effective efficacy endpoint, additional exploratory efficacy endpoints will be described in the SAP.

Continuous variables will be summarized with descriptive statistics of the actual values and the changes from baseline where appropriate. For categorical variables, frequency tables will be presented. Difference in proportions and corresponding confidence intervals (CIs) may be calculated where appropriate.

The relation between viral load and clinical symptoms will be graphically explored.

Spirometry

Descriptive statistics of values and changes from baseline for FEV_1, FVC, and FEV_1/FVC will be summarized at each scheduled timepoint. The percentage of subjects with values beyond clinically important limits will be summarized.

11.5. Immunogenicity Analyses

No formal hypothesis on immunogenicity will be tested. Descriptive statistics (geometric mean and 95% CI for ELISA and virus neutralization assays; median and quartiles for ELISpot and intracellular cytokine staining [ICS]) will be calculated for continuous immunologic parameters at all timepoints. For the humoral assays, geometric mean fold rises from baseline and corresponding 95% CIs may be calculated as well. For immunogenicity, baseline is considered as the last assessment pre-vaccination with Ad26.RSV.preF or placebo. Graphical representations of immunologic parameters will be made as applicable.

Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters as applicable.

The primary analysis set for immunogenicity is the PPI set. As a sensitivity analysis, key tables will also be based on the FA set. Depending on their occurrence, the effect of missed doses or natural infections might be further explored. Note that they will be included in the tables showing the FA set.

11.6. Efficacy versus Immunogenicity Relationship

The relationship between immunogenicity parameters and the VL-AUC as measured by RT-PCR and the AUC for clinical symptoms will be graphically explored.

11.7. Safety Analyses

No formal statistical testing of safety data is planned. All safety data will be analyzed descriptively by regimen.

Adverse Events

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

active phases (ie, AEs emerging after vaccination up to 28 days post-vaccination or during the quarantine phase), and all SAEs, will be included in the analysis. For each AE, the percentage of subjects who experience at least one occurrence of the given event will be summarized by group separately for the vaccination phase and challenge phase.

Summaries, listings, datasets, or subject narratives may be provided, as appropriate, for those subjects who die, who discontinue study vaccine due to an AE, or who experience a severe AE or an SAE.

Summaries and/or listings may be provided separately for AEs with onset outside the active phases.

Solicited local (at injection site) and systemic AEs will be summarized descriptively. The overall frequencies per vaccine group as well as frequencies according to severity and duration will be calculated for solicited AEs. In addition, the number and percentages of subjects with at least one solicited local (at injection site) or systemic AE will be presented. Frequencies of unsolicited AEs, separately for all and vaccination-related only, will be presented by System Organ Class and preferred term. Unsolicited AEs will be presented separately for vaccination phase and quarantine phase.

Clinical Laboratory Tests

Laboratory abnormalities will be determined according to the FDA Guidance document (see Attachment 1), or in accordance with the normal ranges for the clinical laboratory parameter if no grades are available. If a laboratory value falls within the grading as specified in the FDA table, but also within the laboratory normal limits, the value is considered as normal. Emerging abnormalities will be tabulated.

Vital Signs

A tabulation of the distribution of temperatures per half-degree intervals will be provided. For systolic and diastolic blood pressures, heart rate and respiratory rate, the percentage of subjects with values beyond clinically relevant limits will be summarized.

Electrocardiogram

Any abnormalities in ECG parameters (at screening and on admission to the Unit) will be listed.

Physical Examination

Any abnormality in physical examination considered to be clinically significant will be captured as an AE and shown in the AE outputs. Therefore, no separate analysis will be done.

11.8. Planned Analyses

The following analyses will be performed:

- PRIMARY ANALYSIS will be performed when all subjects have completed the quarantine phase or discontinued earlier. The pre-challenge neutralization and pre-F ELISA, and post-challenge RT-PCR, nasal mucous and tissue count, and clinical symptom data will be available although the analysis may be performed on snapshot data. All other data available at the time of primary analysis, including completed cellular data, will be included. Preliminary safety data, including Day 28 post-challenge, if available, will also be included.

- FINAL ANALYSIS will be performed when all subjects have completed the 6 month safety follow-up visit or discontinued earlier.

Depending on when the data are available, both analyses might be combined.

Additional interim analyses (blinded or unblinded) may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner, or upon health authority request. The results will not influence the conduct of the study in terms of early termination or later safety, efficacy or immunogenicity endpoint assessments, and will only be available to a selected group of sponsor personnel, excluding personnel involved in data management and data collection.

11.9. Data Review Committee

Data Review Committee

An internal DRC will be commissioned for this study, comprised of sponsor personnel not directly involved in the conduct of the study, who have expertise in clinical study conduct and vaccines. It will consist of at least one medical expert in the relevant therapeutic area and at least one statistician.

There are no planned DRC reviews. The DRC will only convene to discuss any significant or unexpected safety issues. The investigator and SRP will inform the DRC of any AE of concern.

After such a review, the DRC will make recommendations regarding the continuation of the study. The conclusions of the DRC will be communicated to the investigators, the IRB/IEC and the national regulatory authorities as appropriate. Details will be provided in a separate DRC charter.

If deemed necessary for safety review, the DRC may request the randomization codes and review unblinded data, if applicable.

11.10. Study Vaccination Pausing Rules

The investigator and the SRP will monitor the study vaccination pausing rules. If study vaccination is considered to raise significant safety concerns, further vaccination of subjects will be suspended until DRC review is carried out and subsequent communication between the sponsor and the investigators takes place.

The occurrence of any of the following events will lead to suspension of further study vaccination, and trigger a meeting of the DRC to discuss study suspension, adaptation or discontinuation of further vaccination:

1. One or more subject experience an SAE or other potentially life-threatening (Grade 4) event that is determined to be related to study vaccine; *OR*
2. One or more subjects experience anaphylaxis clearly not attributable to other causes than vaccination with study vaccine; *OR*
3. Two or more subjects experience a Grade 3 or 4 unsolicited AE of the same type, determined to be related to study vaccine, that persists for 72 hours or longer; *OR*
4. Two or more subjects experience a Grade 3 or 4 solicited systemic AE of the same type, determined to be related to study vaccine, that persists for 72 hours or longer; *OR*
5. Two or more subjects experience a persistent (upon repeat testing) Grade 3 or 4 laboratory abnormality related to the same laboratory parameter and considered related to study vaccine, that persists for 72 hours or longer; *OR*
6. Death of any subject, regardless of causality.

If any of the above specific pausing rules are met, the DRC will make recommendations regarding the continuation of the study to the sponsor. Study suspensions or terminations will occur within 5 working days after the decision is made, unless local regulations specify a shorter timeframe. Local regulatory authorities including IECs/IRBs will be informed within the appropriate regulatory-mandated timeframes. A study may be resumed only upon approval of a substantial amendment to the initial study application by the local regulatory authorities and IECs/IRBs. The sponsor will communicate conclusions regarding study continuation to the investigators, the IECs/IRBs and the national regulatory authorities as appropriate.

The investigator may ask for a review meeting to be held for any single event or combination of multiple events which, in his/her professional opinion, jeopardize the safety of the subjects or the reliability of the data.

Vaccinations for the study may be suspended for safety concerns other than those described above or before pausing rules are met if, in the judgment of investigator, subject safety may be threatened. The sponsor should be notified that the DRC will need to be convened.

This is a single site study so stopping rules will automatically apply to the entire study. Sponsor activities and responsibilities related to temporary study suspension and restart are described in the sponsor's applicable standard operating procedures (SOPs).[17,18]

12. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established SOPs in conformity with regulatory

requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. For some studies, subjects are not always able to provide valid verbal responses to open-ended questions. In these circumstances, another method of detecting these events is specified.

Solicited Adverse Events

Solicited AEs are pre-defined local (ie, at the injection site) and systemic events for which subjects are specifically questioned and which are noted by subjects in their diaries (see Section 9.1.1).

Unsolicited Adverse Events

Unsolicited AEs are all AEs for which subjects are specifically not questioned in the subject diary.

12.1. Definitions

12.1.1. Adverse Event Definitions and Classifications

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with study vaccine. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: In this study, the sponsor will collect unsolicited AEs from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge, and solicited AEs from the time of vaccination for 7 days post-vaccination (refer to Section 12.3.1). SAEs will be collected from ICF signature for the duration of the study.

Serious Adverse Event

An SAE based on ICH and European Union (EU) Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study vaccine and the event (eg, death from anaphylaxis), the event must be reported as a suspected unexpected serious adverse reaction (SUSAR) by the sponsor to health authorities and by the investigator to the IRB/IEC according to regulatory and local requirements.

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For Ad26.RSV.preF, the expectedness of an AE will be determined by whether or not it is listed in the Investigator's Brochure.[1]

Adverse Event Associated With the Use of the Vaccine

An AE is considered associated with the use of the vaccine if the attribution is related by the definitions listed in Section 12.1.2.

12.1.2. Attribution Definitions

Every effort should be made by the investigator to explain any AE and assess its potential causal relationship, ie, to administration of the study vaccine, to viral challenge or to alternative causes (eg, natural history of the underlying diseases, concomitant therapy). This applies to all AEs, whether serious or non-serious.

Note: AEs occurring form ICF signature until 28 days after vaccination will be examined for relationship to study vaccine. AEs occurring from admission to the quarantine Unit until 28 days after challenge will be evaluated for relationship to the challenge.

Causality of AEs should be assessed by the investigator based on the following:

Related: there is suspicion that there is a relationship between the study vaccine (or viral challenge) and the AE (without determining the extent of probability); there is a reasonable possibility that the study vaccine (or viral challenge) contributed to the AE.

Unrelated: there is no suspicion that there is a relationship between the study vaccine (or viral challenge) and the AE; there are other more likely causes and administration of the study vaccine (or viral challenge) is not suspected to have contributed to the AE.

By definition, all solicited AEs at the injection site (local) will be considered related to the study vaccine administration.

12.1.3. Severity Criteria

All AEs and laboratory data will be coded for severity using the toxicity grading table in Attachment 1. *Note*: Laboratory values within local laboratory normal ranges (even if within a toxicity grade range), or laboratory values outside normal ranges that are not clinically significant in the judgment of the investigator, should not be recorded as AEs. For AEs not identified in the grading table, the following guidelines will be applied:

Mild (Grade 1): No interference with activity.

Moderate (Grade 2): Some interference with activity not requiring medical intervention.

Severe (Grade 3): Prevents daily activity and requires medical intervention.

Potentially life-threatening (Grade 4): Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability.

The toxicity grading scale used for laboratory assessments is based on the FDA toxicity grading table, consistent with the assessment grading used throughout the protocol. If a laboratory value falls within the grading as specified in the FDA table, but also within the laboratory normal limits, the value is considered as normal. For hemoglobin, both the actual value and the change from reference will be graded.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (eg, laboratory abnormalities).

The severity of solicited AEs will be graded in the diary by the subject based on the severity assessment provided in the diary and then verified by the investigator using the FDA toxicity grading table (see Attachment 1).

12.2. Special Reporting Situations

Safety events of interest on a sponsor study vaccine that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Suspected abuse/misuse of a sponsor study vaccine
- Accidental or occupational exposure to a sponsor study vaccine
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study vaccine, eg, name confusion)
- Exposure to a sponsor study vaccine from breast-feeding

Special reporting situations should be recorded in the eCRF. Any special reporting situation that meets the criteria of an SAE should be recorded on the serious adverse event page of the eCRF.

12.3. Procedures

12.3.1. All Adverse Events

Unsolicited AEs and special reporting situations will be reported from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge.

From challenge until discharge from the Unit, SSC findings greater than Grade 0 will be presumed to represent virus infection consequent to challenge, and will not additionally be captured as an AE, unless they meet the criteria for an SAE. The changes in RSV viral load will not be reported as AEs or SAEs.

Solicited AEs will be recorded by each subject in the subject diary for 7 days after dosing. The investigator will review each subject's diary at the subsequent in-clinic visit; diary information will be transcribed by the study personnel in the on-site assessment forms in the eCRF.

The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

The investigator will monitor and check the study data including all AE and any clinical laboratory data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. All AEs will be deemed related to study vaccine or not related to study vaccine, or related to the viral challenge or not related to viral challenge, according to Section 12.1.2.

The investigator must review both post-injection reactogenicity and other AEs to insure the prompt and complete identification of all events that require expedited reporting as SAEs, invoke pausing rules or are other serious and unexpected events.

All AEs, regardless of seriousness, severity, or presumed relationship to study vaccine, must be recorded using medical terminology in the source document and the eCRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough,

runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). The investigator must record in the eCRF their opinion concerning the relationship of the AE to study vaccine. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational institute where required) all SUSARs. The investigator (or sponsor where required) must report SUSARs to the appropriate IEC/IRB that approved the protocol unless otherwise required and documented by the IEC/IRB. A SUSAR will be reported to regulatory authorities unblinded. Participating investigators and IEC/IRB will receive a blinded SUSAR summary, unless otherwise specified.

Each subject will be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the subject is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical staff only)
- Site number
- Subject number
- Any other information that is required to do an emergency breaking of the blind

12.3.2. Serious Adverse Events

All SAEs occurring from ICF signature until the end of the study must be reported to the appropriate sponsor contact person by study-site personnel within 24 hours of their knowledge of the event.

Information regarding SAEs will be transmitted to the sponsor using the sponsor's Serious Adverse Event Form, which must be completed and signed by a physician from the study site, and transmitted to the sponsor within 24 hours. The initial and follow-up reports of an SAE should be made by facsimile (fax).

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available

- The event can be attributed to agents other than the study vaccine or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as an SAE. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as an SAE, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or an AE (eg, social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the eCRF). *Note*: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered as SAEs. Any AE that results in a prolongation of the originally planned hospitalization is to be reported as a new SAE.

The cause of death of a subject in a study during the entire study period, whether or not the event is expected or associated with the study vaccine, is considered an SAE and must be reported.

12.3.3. Pregnancy

All initial reports of pregnancy in female subjects or partners of male subjects must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the sponsor's appropriate pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies and ectopic pregnancy) are considered SAEs and must be reported using the SAE Form.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported as noted above.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

12.4. Contacting Sponsor Regarding Safety

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed in the Contact Information page(s), which will be provided as a separate document.

13. PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality,

durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

13.1. Procedures

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

If the defect is combined with an SAE, the study-site personnel must report the PQC to the sponsor according to the SAE reporting timelines (refer to Section 12.3.2). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

13.2. Contacting Sponsor Regarding Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed in the Contact Information page(s), which will be provided as a separate document.

14. STUDY VACCINE INFORMATION

14.1. Physical Description of Study Vaccine

A human replication-incompetent adenovirus-vectored vaccine candidate, manufactured and provided under the responsibility of the sponsor, will be assessed in this study:

Ad26.RSV.preF (JNJ-64400141)

Ad26.RSV.preF is a replication-incompetent Ad26 containing a DNA transgene that encodes for the pre-fusion conformation-stabilized F protein derived from the RSV A2 strain.

For this study, Ad26.RSV.preF will be formulated as a solution for intramuscular injection. Ad26.RSV.preF will be supplied as a frozen liquid to be thawed prior to use. Ad26.RSV.preF will be filled in stoppered and sealed 2 mL single-use glass vials in a volume of 0.75 mL to allow an extractable volume of at least 0.5 mL ($1x10^{11}$ vp). Refer to the Investigator's Brochure for details of the components of Ad26.RSV.preF and a list of excipients.[1]

Placebo

Placebo will be supplied as sterile 0.9% saline for injection in 2 mL ampules.

14.2. Packaging and Labeling

All study vaccines were manufactured and packaged in accordance with Current Good Manufacturing Practice. All study vaccines will be packaged and labeled under the responsibility

of the sponsor. Study vaccine labels will contain information to meet the applicable regulatory requirements.

No study vaccine can be repacked or relabeled without prior approval from the sponsor.

Further details for study vaccine packaging and labeling can be found in the Investigational Product Preparation Instructions.

14.3. Storage and Handling

Vials must be stored in a secured location under controlled temperature with no access for unauthorized personnel. The study refrigerator/freezer must be equipped with a continuous temperature monitor and alarm. Study refrigerators/freezers should be equipped with back-up power systems. In the event that study vaccine is exposed to temperatures outside the specified temperature range, all relevant data will be sent to the sponsor to determine if the affected study vaccine can be used or will be replaced. The affected study vaccine must be quarantined and not used until further instruction from the sponsor is received.

Injections should be administered in the non-dominant deltoid.[a] No local or topical anesthetic will be used prior to the injection. The study vaccine will be prepared by the unblinded site pharmacist, or other qualified individual who will have no other study function and administered by a blinded vaccine administrator.

Note: the unblinded pharmacist, or other qualified individual, may also perform the administration, but will have no other study function.

Further details for study vaccine storage, preparation, handling and stability can be found in the Investigational Product Preparation Instructions.

14.4. Vaccine Accountability

The investigator is responsible for ensuring that all study vaccine received at the site is inventoried and accounted for throughout the study. The study vaccine administered to the subject must be documented on the vaccine accountability form. All study vaccine will be stored and disposed of according to the sponsor's instructions.

Study vaccine must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study vaccine must be available for verification by the sponsor's study-site monitor during on-site monitoring visits. The return to the sponsor of unused study vaccine will be documented on the vaccine return form. When the study site is an

[a] For each subject, study vaccine on Day 57 will be administered in the deltoid muscle of the same (non-dominant) arm used for study vaccine administration on Day 1.

authorized destruction unit and study vaccine supplies are destroyed on-site, this must also be documented on the vaccine return form.

Potentially hazardous materials such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for vaccine accountability purposes.

Study vaccine should be dispensed under the supervision of the investigator or a qualified member of the study-site personnel, or by a hospital/clinic pharmacist. Study vaccine will be supplied only to subjects participating in the study. Study vaccine may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study vaccine from, nor store it at, any site other than the study site agreed upon with the sponsor.

15. STUDY-SPECIFIC MATERIALS

The investigator will be provided with the following supplies:

- Investigator's Brochure for Ad26.RSV.preF
- Investigational Product Preparation Instructions/Investigational Product Procedures Manual
- Laboratory Manual
- Trial Center File
- Electronic Data Capture (eDC) Manual/eCRF completion guidelines and randomization instructions
- Sample ICF
- Subject diary
- Subject symptom card
- Ruler
- Thermometers
- Contact information page(s)

16. ETHICAL ASPECTS

16.1. Study-Specific Design Considerations

Potential subjects will be fully informed of the risks and requirements of the study and, during the study, subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled.

The total blood volume drawn from each adult subject will not exceed the US Department of Health and Human Services (HHS) Office for Human Research Protections (OHRP), and FDA guidelines of 550 mL in any eight-week period.[40,41]

See Section 1.3 for the benefit-risk assessment.

16.2. Regulatory Ethics Compliance

16.2.1. Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

16.2.2. Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct, unless required locally), the ICF, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of

this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of AEs that are serious, unlisted/unexpected, and associated with the study vaccine
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study, where required.

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

16.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the subject can read and understand. The

informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the study-site personnel must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive. Finally, they will be told that the investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access. It also denotes that the subject agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

16.2.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

16.2.5. Long-Term Retention of Samples for Additional Future Research

Samples collected in this study may be stored for up to 15 years (or according to local regulations) for additional research. Samples will only be used to understand Ad26.RSV.preF, to understand RSV, and to develop tests/assays related to Ad26.RSV.preF and RSV. The research may begin at any time during the study or the post-study storage period.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers. Subjects may withdraw their consent for their samples to be stored for research (refer to Section 10.2).

17. ADMINISTRATIVE REQUIREMENTS

17.1. Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study, the IEC/IRB (where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative listed in the Contact Information page(s), which will be provided as a separate document. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the eCRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

17.2. Regulatory Documentation

17.2.1. Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

17.2.2. Required Pre-study Documentation

The following documents must be provided to the sponsor before shipment of study vaccine to the study site:

- Protocol and amendment(s), if any, signed and dated by the investigator.

- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, subject compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable.
- Signed and dated statement of investigator (eg, Form FDA 1572), if applicable.
- Documentation of investigator qualifications (eg, curriculum vitae).
- Completed investigator financial disclosure form from the investigator, where required.
- Signed and dated Clinical Trial Agreement, which includes the financial agreement.
- Any other documentation required by local regulations.
- Genetically modified organism (GMO) and/or Institutional Biosafety Committee (IBC) approval, if applicable.

The following documents must be provided to the sponsor before enrollment of the first subject:

- Completed investigator financial disclosure forms from all subinvestigators.
- Documentation of subinvestigator qualifications (eg, curriculum vitae).
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable.
- Local laboratory documentation demonstrating competence and test reliability (eg, accreditation/license), if applicable.

17.3. Subject Identification, Enrollment, and Screening Logs

The investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by subject identification

and date of birth. In cases where the subject is not randomized into the study, the date seen and date of birth will be used.

The investigator must also complete a subject screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

17.4. Source Documentation

At a minimum, source documents consistent in the type and level of detail with that commonly recorded at the study site as a basis for standard medical care must be available for the following: subject identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and immunogenicity parameters as required by the protocol; record of all AEs and follow-up of AEs; concomitant medication; vaccine receipt/dispensing/return records; study vaccine administration information; and date of study completion and reason for early discontinuation of study vaccine or withdrawal from the study, if applicable.

The author of an entry in the source documents should be identifiable.

Specific details required as source data for the study and source data collection methods will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The subject diary used to collect information regarding solicited events after vaccination will be considered source data. At the visit at 7 days after vaccination, information from the subject diary will be reviewed by the investigator; diary information will be transcribed by study personnel into the eCRF as described in the eCRF Completion Guidelines.

An eSource system may be utilized, which contains data traditionally maintained in an hospital or clinic record to document medical care (eg, electronic source documents) as well as the clinical study-specific data fields as determined by the protocol. This data is electronically extracted for use by the sponsor. If eSource is utilized, references made to the eCRF in the protocol include the eSource system but information collected through eSource may not be limited to that found in the eCRF.

17.5. Case Report Form Completion

Case report forms are prepared and provided by the sponsor for each subject in electronic format. All eCRF entries, corrections, and alterations must be made by the investigator or authorized study-site personnel. The investigator must verify that all data entries in the eCRF are accurate and correct.

The study data will be transcribed by study-site personnel from the source documents onto an eCRF, if applicable. Study-specific data will be transmitted in a secure manner to the sponsor.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the subject's source documents. Data must be entered into eCRF in English. The eCRF must be completed as soon as possible after a subject visit and the forms should be available for review at the next scheduled monitoring visit.

If necessary, queries will be generated in the eDC tool. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in either of the following ways:

- Investigator and study-site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Sponsor or sponsor delegate can generate a query for resolution by the investigator and study-site personnel.

17.6. Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, and periodic monitoring visits by the sponsor. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for eCRF completion will be provided and reviewed with study-site personnel before the start of the study. The sponsor will review eCRF for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

17.7. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eCRF and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will

accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

17.8. Monitoring

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study-site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the eCRF with the source documents (eg, hospital/clinic/physician's office medical records). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to source documents (medical records) must be allowed for the purpose of verifying that the recorded data are consistent with the original source data. Findings from this review will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documents will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

17.9. Study Completion/Termination

17.9.1. Study Completion/End of Study

The end of the study will be the last subject's last visit by telephone at 6 months post-vaccination. The study is considered completed with the last visit for the last subject participating in the study. The final data from the study site will be sent to the sponsor (or designee) after completion of the final subject visit at that study site, in the time frame specified in the Clinical Trial Agreement.

17.9.2. Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. The study site will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study vaccine development

The study will be terminated if one or more subjects experience any severe or clinically significant illness from RSV challenge, after discussion with the investigator, hVIVO, and the sponsor's SRP.

17.10. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection. Subject privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

17.11. Use of Information and Publication

All information, including but not limited to information regarding Ad26.RSV.preF or the sponsor's operations (eg, patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor in connection with the continued development of Ad26.RSV.preF, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the sponsor and will contain data from all study sites that participated in the study as per protocol. Recruitment

performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator. Results of analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report. Study subject identifiers will not be used in publication of results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the sponsor as author and owner of copyright in such work.

Consistent with Good Publication Practices and International Committee of Medical Journal Editors guidelines, the sponsor shall have the right to publish such primary (multi-center) data and information without approval from the investigator. The investigator has the right to publish study site-specific data after the primary data are published. If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to the sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the sponsor in writing, the investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the sponsor will review these issues with the investigator. The sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multi-center study designs and substudy approaches, secondary results generally should not be published before the primary endpoints of a study have been published. Similarly, investigators will recognize the integrity of a multi-center study by not submitting for publication data derived from the individual study site until the combined results from the completed study have been submitted for publication, within 12 months of the availability of the final data (tables, listings, graphs), or the sponsor confirms there will be no multi-center study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and disclose the existence of and the results of clinical studies as required by law.

REFERENCES

1. Investigator's Brochure: Prophylactic RSV Vaccine. Edition 5. Janssen Vaccines & Prevention B.V.
2. Abel B, Tameris M, Mansoor N, et al. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. Am J Respir Crit Care Med. 2010:181;1407-1417.
3. Anderson LJ, Dormitzer PR, Nokes DJ, et al. Strategic priorities for respiratory syncytial virus (RSV) vaccine development. Vaccine. 2013:31 Suppl 2;B209-215.
4. Baden LR, Walsh SR, Seaman MS, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). J Infect Dis. 2013:207;240-247.
5. Barouch DH, Liu J, Peter L, et al. Characterization of humoral and cellular immune responses elicited by a recombinant adenovirus serotype 26 HIV-1 Env vaccine in healthy adults (IPCAVD 001). J Infect Dis. 2013:207;248-256.
6. Centers for Disease Control and Prevention. Respiratory Syncytial Virus Infection (RSV) - Infection and Incidence. Available at: http://www.cdc.gov/rsv/about/infection.html (last accessed 05 January 2017).
7. Creech CB, Dekker CL, Ho D, et al. Randomized, placebo-controlled trial to assess the safety and immunogenicity of an adenovirus type 35-based circumsporozoite malaria vaccine in healthy adults. Hum Vaccin Immunother. 2013:9;2548-2557.
8. DeVincenzo JP, Whitley RJ, Mackman RL, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N Engl J Med. 2014:371;711-722.
9. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. Am J Respir Crit Care Med. 2010:182;1305-1314.
10. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med. 2005:352;1749-1759.
11. Falsey AR, Walsh EE. Respiratory syncytial virus infection in adults. Clin Microbiol Rev. 2000:13;371-384.
12. Gidudu JF, Walco GA, Taddio A, et al. Immunization site pain: case definition and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2012:30;4558-4577.
13. Gilman MS, Castellanos CA, Chen M, et al. Rapid profiling of RSV antibody repertoires from the memory B cells of naturally infected adult donors. Sci Immunol. 2016:1;
14. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child. 1986:140;543-546.
15. Graham BS, Modjarrad K, McLellan JS. Novel antigens for RSV vaccines. Curr Opin Immunol. 2015:35;30-38.
16. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med. 2009:360;588-598.
17. Janssen Research and Development. Clinical Trial Termination, Suspension or Hold, or Amendment for Safety Reasons. Standard Operating Procedure: TV-SOP-07839.
18. Janssen Research and Development. Temporary Suspension, Premature Study Termination, and Study Closure. Standard Operating Procedure: TV-SOP-04045.
19. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am J Epidemiol. 1969:89;405-421.
20. Kohl KS, Walop W, Gidudu J, et al. Induration at or near injection site: case definition and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2007:25;5839-5857.
21. Kohl KS, Walop W, Gidudu J, et al. Swelling at or near injection site: case definition and guidelines for collection, analysis and presentation of immunization safety data. Vaccine. 2007:25;5858-5874.
22. Krarup A, Truan D, Furmanova-Hollenstein P, et al. A highly stable prefusion RSV F vaccine derived from structural analysis of the fusion mechanism. Nat Commun. 2015:6;8143.

23. Magro M, Mas V, Chappell K, et al. Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention. Proc Natl Acad Sci U S A. 2012:109;3089-3094.

24. Marcy SM, Kohl KS, Dagan R, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004:22;551-556.

25. Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia ankara-vectored ebola vaccines: a randomized clinical trial. JAMA. 2016:315;1610-1623.

26. Moghaddam A, Olszewska W, Wang B, et al. A potential molecular mechanism for hypersensitivity caused by formalin-inactivated vaccines. Nat Med. 2006:12;905-907.

27. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. 2010:375;1545-1555.

28. Ngwuta JO, Chen M, Modjarrad K, et al. Prefusion F-specific antibodies determine the magnitude of RSV neutralizing activity in human sera. Sci Transl Med. 2015:7;309ra162.

29. Ouedraogo A, Tiono AB, Kargougou D, et al. A phase 1b randomized, controlled, double-blinded dosage-escalation trial to evaluate the safety, reactogenicity and immunogenicity of an adenovirus type 35 based circumsporozoite malaria vaccine in Burkinabe healthy adults 18 to 45 years of age. PLoS One. 2013:8;e78679.

30. Paramore LC, Ciuryla V, Ciesla G, Liu L. Economic impact of respiratory syncytial virus-related illness in the US: an analysis of national databases. Pharmacoeconomics. 2004:22;275-284.

31. Peebles RS, Jr. Viral infections, atopy, and asthma: is there a causal relationship? J Allergy Clin Immunol. 2004:113;S15-18.

32. Radosevic K, Rodriguez A, Lemckert AA, et al. The Th1 immune response to Plasmodium falciparum circumsporozoite protein is boosted by adenovirus vectors 35 and 26 with a homologous insert. Clin Vaccine Immunol. 2010:17;1687-1694.

33. Regnier SA, Huels J. Association between respiratory syncytial virus hospitalizations in infants and respiratory sequelae: systematic review and meta-analysis. Pediatr Infect Dis J. 2013:32;820-826.

34. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980-1996. JAMA. 1999:282;1440-1446.

35. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med. 2005:171;137-141.

36. Simoes EA, Carbonell-Estrany X, Rieger CH, et al. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. J Allergy Clin Immunol. 2010:126;256-262.

37. Simoes EA, Groothuis JR, Carbonell-Estrany X, et al. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. J Pediatr. 2007:151;34-42, 42 e31.

38. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997-2006. Pediatr Infect Dis J. 2012:31;5-9.

39. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA. 2003:289;179-186.

40. US Department of Health and Human Services. Office for Human Research Protections - OHRP Expedited Review Categories (1998). Available at: http://www.hhs.gov/ohrp/regulations-and-policy/guidance/categories-of-research-expedited-review-procedure-1998/index.html (last accessed 05 January 2017).

41. US Food and Drug Administration. Conditions for IRB Use of Expedited Review. Available at: http://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/GuidancesInformationSheetsandNotices/ucm118099.htm (last accessed 05 January 2017).

42. Data on file.

Attachment 1: Toxicity Tables

Adapted From the FDA Guidance document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007)

A: Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to Touch	Discomfort with Movement	Significant discomfort at rest	ER visit or Hospitalization
Erythema/redness*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Fever** (°C) Fever** (°F)	37.8 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	>40 >104
Tachycardia - beats per minute	101 – 115	116 – 130	>130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) - mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate - breaths per minute	17 – 20	21 – 25	>25	Intubation

* Subject should be at rest for all vital sign measurements.

** Oral temperature; no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 - 3 loose stools or <400 gms/24 hours	4 - 5 stools or 400 - 800 gms/24 hours	6 or more watery stools or >800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Systemic Illness	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life-threatening (Grade 4)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

B: Tables for Laboratory Abnormalities

The grading scale used for laboratory assessments is based on the FDA Guidance document "Toxicity Grading Scale from Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". Any laboratory value shown as a "graded" value in the table that is within the local laboratory normal ranges will not be graded for severity or recorded as AE. For hemoglobin, both the actual value and the change from reference will be graded. For the change from reference, the corresponding actual value should also be at least Grade 1.

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4) **
Sodium – Hyponatremia - mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia - mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia - mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia - mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia - mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting - mg/dL	100 – 110	111 – 125	> 125	Insulin requirements or hyperosmolar coma
Random Glucose - mg/dL	110 – 125	126 – 200	>200	
Blood Urea Nitrogen BUN - mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine - mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia - mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia - mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia - mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia - mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK - mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia - g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4) **
Total Protein – Hypoproteinemia - g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests – ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	--
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

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

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

***ULN is the upper limit of the normal range.

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4) **
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm3	10,800 – 15,000	15,001 – 20,000	20,001 – 25,000	> 25,000
WBC Decrease - cell/mm3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm3	650 – 1500	1501 – 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT - increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT - increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

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

** ULN is the upper limit of the normal range.

Attachment 2: Objectives and Endpoints Table

Objectives	*Endpoints*
PRIMARY	
• The primary objective is to assess a trend for the prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly to adults aged 18-50 years in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the AUC over time by quantitative RT-PCR compared to placebo.	• VL-AUC of RSV-A Memphis 37b, determined by quantitative RT-PCR assay of nasal wash samples.
SECONDARY	
• To assess a trend for prophylactic efficacy of a single dose of Ad26.RSV.preF in the RSV challenge model in terms of reduction of nasal wash viral load as measured by the peak viral load of the RT-PCR compared to placebo.	• Peak viral load of RSV-A Memphis 37b, defined as the maximum viral load as determined by quantitative RT-PCR assay of nasal wash samples, observed over the entire time period.
• To assess the effect of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF on viral load and clinical symptoms on Day 6 and Day 7 post-challenge compared to placebo.	• Viral load, measured by RT-PCR and quantitative culture of RSV, and clinical symptoms on Day 6 and Day 7.
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection and one or more positive clinical symptoms of any grade from two different categories in the symptom scoring system, or one Grade 2 symptom from any category compared to placebo.	• Occurrence of symptomatic RSV infection plus symptoms of any grade from two different categories from the SSC or two quantifiable RT-PCR measurements plus any Grade 2 symptom from any category. The three SSC categories are: o Upper Respiratory symptoms: runny nose, stuffy nose, sneezing, sore throat, earache o Lower Respiratory symptoms: cough, shortness of breath, chest tightness, wheeze o Systemic symptoms: malaise, headache, muscle and/or joint ache, chilliness/feverishness.
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection and one or more positive clinical symptoms of any grade in any category from the symptom scoring system compared to placebo.	• Occurrence of RSV infection plus any clinical symptom of any severity.
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of the weight of mucous secretions and tissue count over time compared to placebo.	• Total weight of mucus produced and tissue count.
• To assess the safety and tolerability of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF administered intramuscularly.	• SAEs throughout the study (from signing the ICF to the end of the study, 6 months after vaccination).

Objectives	*Endpoints*
	• Solicited local and systemic AEs for 7 days after vaccination. • Unsolicited AEs from ICF signature until 28 days after vaccination, and from the entry into the Unit until 28 days after challenge. • Safety data including, but not limited to, physical examinations, vital signs, 12-lead ECGs and clinical laboratory results (including biochemistry, hematology, and urinalysis).
EXPLORATORY	
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of clinical symptoms as measured by the AUC over time of symptoms collected by a graded symptom scoring system compared to placebo.	• AUC of the total clinical symptom score. The total clinical symptom score is a composite of 13 self-reported symptoms (on the SSC) divided into three categories (Upper Respiratory, Lower Respiratory, Systemic).
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of VL-AUC compared to placebo.	• VL-AUC of RSV-A Memphis 37b, determined by quantitative culture of RSV of nasal wash samples.
• To explore the relationship of humoral and cellular immunogenicity outcomes (including nasal wash if feasible) and the VL-AUC and the AUC for clinical symptoms.	• AUC of the total clinical symptom score. • VL-AUC of RSV-A Memphis 37b. • RSV pre-F and post-F antibodies from serum and nasal wash samples[a] measured by ELISA. • Cellular immunity measured by IFNγ ELISpot assay, flow cytometry cytokine analysis and/or secreted cytokines.
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of prevention of symptomatic RSV infection and/or positive serological measurement of RSV infection in the context of immunization and pre-existing antibody and one or more positive clinical symptoms of any grade in any category compared to placebo.	• Symptoms of any grade from any categories of the SSC. • Proportion of subjects with symptomatic RSV infection (RT-PCR or serology). • Neutralizing antibody against RSV-Memphis 37b (challenge strain) and RSV A2.
• To assess a trend for prophylactic efficacy of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF in the RSV challenge model in terms of respiratory parameters measured by spirometry during the study compared to placebo.	• Respiratory parameters: spirometry (FEV1, FVC, FEV1/FVC).
• To explore the relationships between clinical symptoms and viral load, as measured by RT-PCR and quantitative culture of RSV, in	• RT-PCR and quantitative culture of RSV. • Symptoms of any grade from any categories of the SSC.

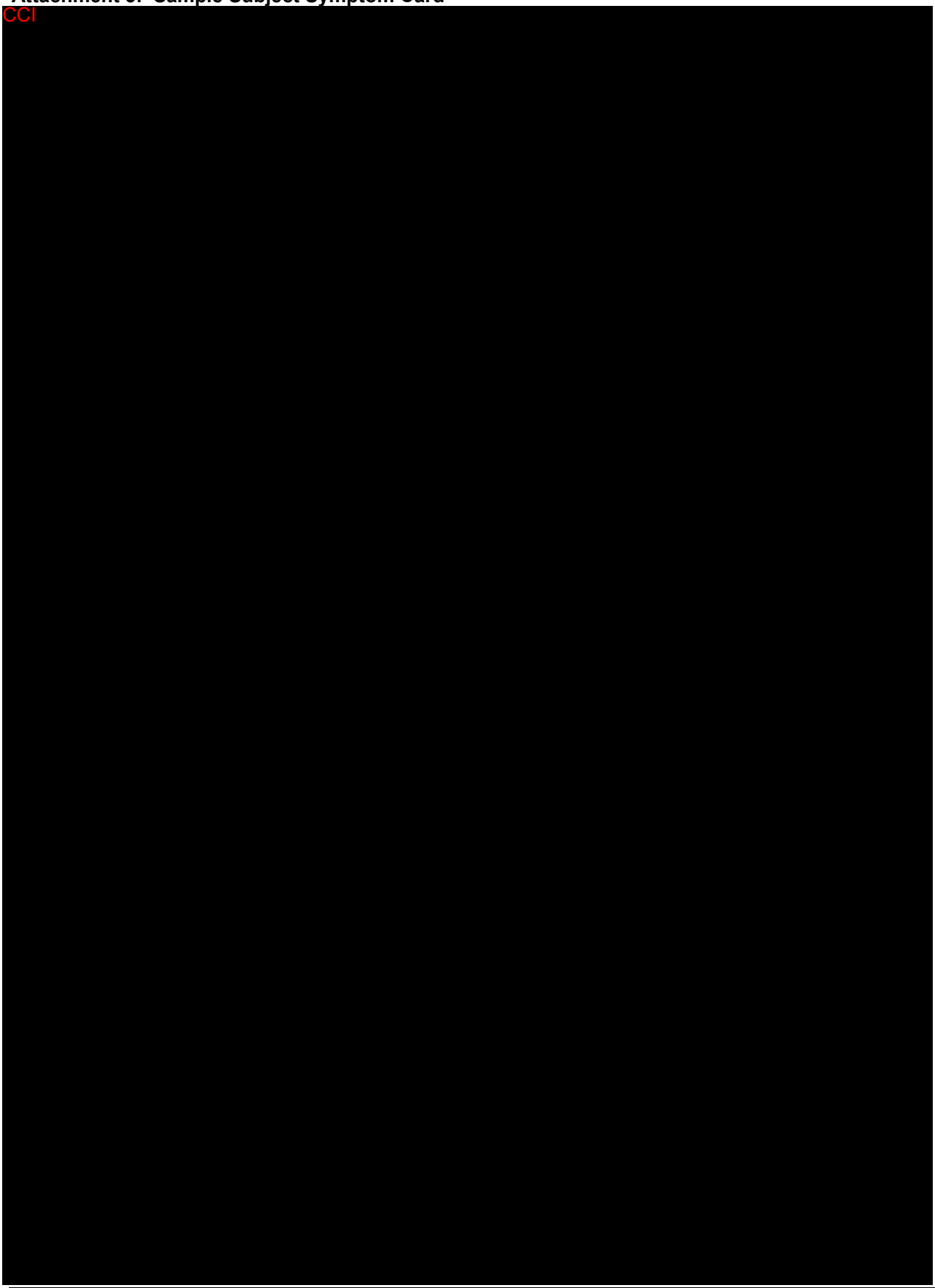
[a] Assumes that the ELISA for nasal wash samples will be available.

Objectives	*Endpoints*
Ad26.RSV.preF subjects and placebo.	
• To explore other immunologic parameters in relation to the VL-AUC by RT-PCR and AUC for clinical symptoms and occurrence of symptomatic RSV infection or more plus one clinical symptom of any grade.	• RT-PCR and quantitative culture of RSV. • VL-AUC of RSV-A Memphis 37b. • Symptoms of any grade from any categories of the SSC. • AUC of the total clinical symptom score.
• To explore the immune response of a single dose of $1x10^{11}$ vp of Ad26.RSV.preF compared to placebo.	• Evaluation of vaccine-specific responses before challenge by F-protein specific humoral responses measured by binding and/or VNA, and increase in cellular responses as measured by ELISpot and/or ICS.
• To explore humoral and cellular responses to challenge with RSV-A Memphis 37b in immunized and non-immunized subjects.	• ELISA (pre-F, total), neutralization, cellular (IFN ELISpot, flow cytometry.
• Additional exploratory analyses may be performed to investigate vaccine-elicited immune responses further.	• RSV cross-neutralization of B and/or other A strain. • F-protein antibody specificity characterization. • Adenovirus neutralization assay. • Functional and molecular antibody characterization.
• To explore other potential efficacy endpoints, as defined in the SAP, to determine the best efficacy endpoint to use in subsequent clinical studies investigating the vaccine's efficacy.	

Note: Symptomatic RSV infection is defined as two quantifiable RT-PCR measurements at different timepoints

Attachment 3: Sample Subject Symptom Card

CCI



Attachment 4: Expected Symptoms after Challenge with the Memphis RSV Strain

The following are expected symptoms after viral challenge and should not be classified as AEs:

Runny nose, stuffy nose, sneezing, sore throat, ear ache, malaise, headache, muscle and/or joint ache, chilliness/feverishness, cough, chest tightness, shortness of breath and wheeze.

Any symptom not in the above list would be an unusual observation and should be recorded as an AE at the discretion of the PI.

INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study vaccine, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): ADRIAN CAPLANUSI, MD

Institution: Janssen Vaccines & Prevention B.V.

Signature: electronic signature appended at the end of the protocol Date: _____

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

LAST PAGE

SIGNATURES

Signed by	Date	Justification
Adrian Caplanusi	24Jul2018, 13:51:52 PM, UTC	Document Approval