Official Title: A Phase I Randomized, Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability and Immunogenicity of a Stabilized Prefusion RSV F Subunit Protein Vaccine, VRC-RSVRGP084-00-VP (DS-Cav1), Alone or with Alum Adjuvant in Healthy Adults

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VACCINE RESEARCH CENTER

Protocol VRC 317
(NIH 17-I-0058)

A PHASE I RANDOMIZED, OPEN-LABEL CLINICAL TRIAL TO EVALUATE DOSE, SAFETY, TOLERABILITY AND IMMUNOGENICITY OF A STABILIZED PREFUSION RSV F SUBUNIT PROTEIN VACCINE, VRC-RSVRGP084-00-VP (DS-CAV1), ALONE OR WITH ALUM ADJUVANT, IN HEALTHY ADULTS

Vaccine Provided by:
National Institute of Allergy and Infectious Diseases
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Clinical Trial Sponsored by:
National Institute of Allergy and Infectious Diseases
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ABBREVIATIONS

Abbreviation	Term
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AoU	assessment of understanding
AST	aspartate aminotransferase
β-HCG	human chorionic gonadotropin
Cav	Cavity Filling Variant of RSV F protein
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CHIKV	chikungunya virus
CI	confidence interval
CMV	cytomegalovirus
CRO	contract research organization
DENV	dengue virus
DNA	deoxyribonucleic acid
DS-Cav1	Disulfide Bond, Cavity-Filling (Variant Molecule) 1 of RSV F protein
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent assay
ELISpot assay	enzyme-linked immunospot assay
F	fusion protein
FDA	Food and Drug Administration
G	glycoprotein
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRPP	Human Research Protections Program
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IM	intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
mAb	monoclonal antibody
mcg	microgram
MedDRA®	Medical Dictionary for Regulatory Activities
mL	milliliter
N	nucleoprotein

Abbreviation	Term
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH CC	National Institutes of Health Clinical Center
NVITAL	NIAID Vaccine Immune T-Cell and Antibody Laboratory
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RSV	Respiratory syncytial virus
SAE	serious adverse event
SARS	Severe acute respiratory syndrome
SH	small hydrophobic protein
SST	serum separator tube
TCR	tissue cross reactivity
ULN	upper limit of normal
UP	unanticipated problem
VE	vaccine efficacy
VIP	Vaccine Immunology Program
VITL	Vaccine Immunology Testing Laboratory
VLP	virus-like particle
VRC	Vaccine Research Center
WBC	white blood cell
WHO	World Health Organization

PRÉCIS

Study Title: A Phase I Randomized, Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability and Immunogenicity of a Stabilized Prefusion RSV F Subunit Protein Vaccine, VRC-RSVRGP084-00-VP (DS-Cav1), Alone or with Alum Adjuvant in Healthy Adults

Study Design: This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of VRC-RSVRGP084-00-VP alone or with alum adjuvant in a 2-injection regimen. The hypotheses are that the vaccine will be safe and tolerable for human administration, and will induce detectable immune response. The primary objective is to evaluate the safety and tolerability of the investigational vaccine at 3 dose levels administered alone or with alum adjuvant as a homologous boost in healthy adults. Secondary objectives relate to humoral and cellular immunogenicity of the investigational vaccine regimen.

Product Description: VRC-RSVRGP084-00-VP (DS-Cav1) was developed by VRC, NIAID and is composed of the respiratory syncytial virus (RSV) fusion (F) glycoprotein ectodomain assembled as a trimer stabilized in its prefusion native conformation with a foldon trimerization domain at the C-terminus and 4 internal mutations designated DS-Cav1 (4.1DHFR_RSVAF). The sequence is based on the RSV A2 strain from subtype A. The product is provided at a concentration of 0.5 mg/mL in 3 mL glass vials filled to 1.2 mL.

Adjuvant is an aluminum hydroxide suspension (alum) provided in a sterile, pyrogen-free suspension at a concentration of 5 mg/mL in 3 mL glass vials filled to 0.7 ± 0.10 mL. The alum dose is 500 mcg and will be field mixed.

Subjects: Healthy adult subjects ages 18-50.

Study Plan: Subject will be randomized into DS-Cav1 or DS-Cav1 plus alum in each dose during the study. Dose continuation and dose escalation evaluations will occur to ensure the safety data support proceeding to the higher doses. Subjects will be evaluated for safety and immune responses through blood and mucosal sample collection at specified timepoints throughout the study. The study schema is below:

VRC 317 Study Schema

Group	Subjects	Dose	Day 0	Week 12[1]
1	15	50 mcg	DS-Cav1	DS-Cav1
2	15		DS-Cav1 + alum	DS-Cav1 + alum
3	15	150 mcg	DS-Cav1	DS-Cav1
4	15		DS-Cav1 + alum	DS-Cav1 + alum
5	15	500 mcg	DS-Cav1	DS-Cav1
6	15		DS-Cav1 + alum	DS-Cav1 + alum
Total	90*	All DS-Cav1 vaccinations are administered with needle and syringe into the deltoid muscle. *Up to 100 subjects may be enrolled if needed to evaluate safety or immunogenicity. [1] This dose is optional for the last 5 subjects enrolled in each group that receive the Day 0 injection and any additional subjects needed to evaluate safety or immunogenicity.		

Duration: The study schedule requires 13 clinic visits and a telephone contact after each injection.

1. INTRODUCTION

Respiratory syncytial virus (RSV) causes upper and lower respiratory infections in children and adults of all ages, and leads to significant morbidity and mortality in pediatric, immunocompromised, and geriatric populations [1]. Worldwide, RSV is the leading cause of respiratory tract infections in children, infecting 50-70% of all children by age one and nearly all children by age two [2, 3]. About 2% of all children will require hospitalization for RSV. Infants born prematurely or those with congenital heart disease have a particularly high risk for severe disease, but more than half of hospitalized infants, have no identified risk factors. The peak age of hospitalization is between 2 and 3 months of age, but more than half of all hospitalizations in children under 5 years of age occur after 6 months [4-7].

Other risk factors include a family history or genetic predisposition to allergic inflammation and increase in IL-13 and IL-4 responses or a low level of maternally-transferred RSV-specific neutralizing activity [8]. Additionally, infants hospitalized for RSV are at greater risk for childhood wheezing and asthma. Adults with compromised immune systems and those ages 65 and older are also at increased risk of severe disease [9, 10].

Nearly all humans contract RSV by the age of three and then repeatedly throughout their lifetime [11, 12]. The economic impact of RSV in the United States (US) alone exceeds $400 million per year [13, 14] and worldwide, annual RSV hospitalizations are estimated to number more than 3.4 million [15].

Currently, there is no licensed RSV vaccine. A single licensed antiviral therapy, Palivizumab (Synagis™), is currently available for prevention of RSV. Palivizumab is an anti-RSV fusion protein-specific monoclonal antibody (mAb) used as passive prophylaxis to reduce the frequency of severe RSV disease in premature infants, but is only recommended for infants born at 28 weeks of gestational age or less [16-20]. Target populations for an RSV vaccine include pregnant women, infants, young children, and the elderly. It is anticipated that age-specific vaccine strategies will be needed to generate effective immunity across a range of age groups [21].

Vaccines to prevent RSV mediated disease are both urgently needed and uniquely challenging. In the late 1960s, a formalin-inactivated RSV vaccine (FI-RSV) was associated with enhanced disease following subsequent RSV infection [22]. Among FI-RSV vaccine-immunized infant subjects (n=31), 80% required hospitalization following infection and two deaths were reported. This is compared to 5% requiring hospitalization following infection and no deaths among the parainfluenza vaccine-immunized control infants (n=40). Severe RSV disease was found to be more frequent in vaccinees than in the same-age general population not enrolled in the vaccine study [23-25]. Because infants and small children are a primary target population for an effective RSV vaccine, the legacy from that trial represents a substantive obstacle for development of an RSV vaccine for antigen-naïve infants.

Unique properties of RSV have provided challenges to the development of a successful vaccine. RSV encodes three envelope proteins, a small hydrophobic (SH) protein of unknown function, an attachment glycoprotein (G), and a fusion (F) protein. Of these proteins, only the F protein is essential for infecting cells *in vitro*; although, *in vivo*, G protein expression provides an advantage for infection of human airway epithelia [26]. The G protein is more variable than any other RSV protein and a high proportion of G is secreted through an alternative translation

initiation site, and acts as an immunomodulatory decoy for adaptive immune responses [27]. Non-structural RSV gene products, NS1 and NS2, both interfere with innate immune responses through cooperative inhibition of interferon induction and effector functions [28, 29].

RSV lacks an optimal animal model. Rodent models such as mice and cotton rats are only semi-permissive for RSV infection, although they are useful for defining immune response patterns and rank-ordering candidate vaccines for neutralizing potency and protective capacity. Nonhuman primates (NHP) are useful for immunogenicity and challenge studies, but possess variable levels of susceptibility to infection and generally require large inocula intranasally and intratracheally to consistently establish infection.

Substantive advances in understanding both RSV biology and immune system development and function have resulted in a number of RSV vaccine development approaches using a variety of platforms, many of which have advanced to clinical evaluation, including live-attenuated vaccines, chimeric virus vaccines and viral-vectored vaccines, usually expressing F, sometimes in combination with other viral genes [30-33]. However, none of these designs based on F have yet demonstrated vaccine efficacy in clinical trials, despite showing protection in animal models, emphasizing the need to use an antigen that induces immune responses relevant to human protection.

Several trials with RSV subunit vaccines have been conducted in pre-immune children and adults. A series of adjuvanted RSV F protein candidate vaccines administered alone or in combination with G and/or *N* (nucleoprotein) were deemed safe in seropositive subjects, but did not generate immune responses of sufficient breadth or potency to advance into antigen-naïve infants [34-38]. Another subunit vaccine composed of a fusion of a protein G fragment to the albumin-binding domain of streptococcal protein G elicited robust immune responses in seropositive humans, but subjects reported an unacceptable rate of erythema purpura [39, 40]. A version of RSV F protein in the post-fusion conformation formulated without adjuvant has advanced to Phase 2 trials. Among 1600 subjects age 60 years or older, 44% effectiveness in preventing all RSV symptoms, 64% effectiveness in preventing lower respiratory tract symptoms, and a 4.8-fold increase in anti-F IgG response among vaccinees was observed [41].

1.1. Rationale for Evaluation of DS-Cav1

Based on the reduction of severe disease by palivizumab, which is specific for the RSV F glycoprotein and studies of maternal cord serum, there is evidence that neutralizing activity in serum is a correlate of protection against RSV. Palivizumab is recommended for prophylactic administration to high-risk neonates during their first winter season, at the height of RSV infection risk [42]. The RSV F protein, like multiple other Class I viral fusion proteins, undergoes a dramatic structural rearrangement as it mediates the membrane fusion process required for viral entry. During this process, it transitions from a metastable prefusion to stable postfusion conformation. By protein adsorption and competition studies, the prefusion conformation of RSV F (pre-F) has been demonstrated to elicit the majority of neutralizing activity in human serum [43, 44]. Knowledge of these key facts led the Vaccine Research Center (VRC) to pursue a structure-guided design approach to preserve neutralization-sensitive sites on pre-F surfaces and produce a stabilized pre-F subunit candidate vaccine.

The co-crystal structure of the RSV F protein and the D25 mAb revealed a previously-unidentified, prefusion-specific quaternary antigenic site, designated antigenic site zero (Ø) that

is abolished in the shift to the stable postfusion conformation. Computational modelling studies undertaken on the basis of this crystal structure led to experiments that evaluated more than 100 combinations of those sequence changes projected to stabilize F in the prefusion conformation. A variant comprising S155C, S290C, S190F, and V207L within the context of wild type F protein sequence, including native C-terminus, met criteria for expression of the F protein stabilized in the prefusion conformation. The two cysteines (S155C, S290C) form a disulfide bond, the two bulky side chain mutations (S190F, V207L) occlude a hydrophobic cavity and promote hydrophobic packing interactions, and together these changes enable expression of an unprecedented conformationally-stable F prefusion protein, DS-Cav1 (Di-Sulfide; Cavity, first variant) [45, 46]. The protein assumed a quaternary structure closely approximating the previously-solved D25-bound RSV F, bound prefusion-specific antibodies with nanomolar to subnanomolar affinities, and in both mouse and NHP vaccine studies, elicited neutralizing titers between 70- and 80-fold greater than those elicited by postfusion F. The majority of antibodies induced by DS-Cav1 are specific for the unique surfaces on prefusion F and the rest target surfaces shared between pre-F and post-F conformations. Because the large majority of RSV neutralizing antibodies in human sera recognize surfaces unique to pre-F, it is anticipated that boosting adults with DS-Cav1 will elicit a greater boost in neutralization activity than prior RSV candidate vaccines that have utilized F antigens in the postfusion conformation.

Based on its safety, immunogenicity and efficacy profiles in preclinical studies [45], the candidate DS-Cav1 vaccine is being evaluated for the first time in healthy adults in this Phase 1 clinical trial. Because preliminary data suggest immune responses after the first dose alone may provide valuable data regarding the duration of the vaccine-induced immune response, the last 5 subjects enrolled into each group will have the option to receiving (or not receiving) the second vaccine dose.

1.2. Rationale for Use of Aluminum–Based Adjuvant

Adjuvants improve the immune response to many vaccines [47, 48]. The most common aluminum-based adjuvants includes aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate, or mixed aluminum salts [49, 50]. Several phenomena contribute to the effect of aluminum hydroxide based adjuvants including: a ‘repository effect’ that occurs when the antigens aggregate on the adjuvant particle and are deposited to the immune cell for long duration to induce immune responses, a pro-phagocytic effect, and possibly the activation of the pro-inflammatory nucleotide like receptor protein 3 dependent pathways [50]. Aluminum-based adjuvants often improve humoral and innate responses and may lead to increased antibody titers, rapid induction of responses, reduction in size or frequency of doses, increased breadth of responses to overcome pathogen diversity, induction of long-lasting immune memory responses, and induction of response to overcome poor immune systems in elderly and young children [51-54]. Based on over six decades of use, aluminum is broadly accepted to be safe, well-tolerated and effective [51, 52].

1.3. Previous Human Experience

The only human experience with VRC-RSVRGP084-00-VP alone or with adjuvant is from the current trial that is ongoing. As of August 08, 2019 a total of 95 subjects have enrolled and received one or two injections of a 50, 150, or 500 mcg DS-Cav1 vaccine, alone or with alum.

All dose escalation steps have been completed as per protocol; and no stopping or pausing rules have been met at any time during the study.Overall, all formulations of the DS-Cav1 vaccine have been well-tolerated and assessed as safe by the Protocol Safety Review Team (PSRT). Immunogenicity data reveal a more than 10-fold boost in neutralizing activity in serum from antibodies targeting prefusion-specific surfaces of RSV F [55].

Aluminum adjuvants have been used in vaccines for many decades with a demonstrated safety profile [56]. Aluminum is the most common adjuvant used in human vaccines licensed by the Food and Drug Administration (FDA) [57, 58]. Aluminum-containing vaccines have been associated with erythema, subcutaneous nodules, contact hypersensitivity, granulomatous inflammation [56, 59]. A specific limitation is neurotoxicity in patients with decreased renal function [57].

Aluminum-based adjuvants promote strong humoral immune responses, and therefore, are incorporated in vaccines against diseases where neutralizing antibodies are required for protection such as diphtheria, tetanus and hepatitis B [54]. The licensed HPV VLP vaccines, Gardasil (Merck & Co, Inc.) and Cervarix (GlaxoSmithKline) have aluminum in the formulations at 0.5 mg of aluminum hydroxyphosphate sulphate per dose and 0.5 mg of aluminum hydroxide per dose, respectively [60].

As per 21 Code of Federal Regulations (CFR) part 610.15, the amount of aluminum in biological products cannot exceed 0.85 mg/dose. The amount of aluminum in vaccines currently licensed in the US ranges from 0.125-0.85 mg/dose [58]. Based on FDA regulations, experience with HPV VLP vaccines and VRC pre-clinical data, VRC plans to use 0.5 mg/dose of aluminum hydroxide adjuvant in this phase 1 study.

1.4. Assessment of Immunogenicity

Specimens to evaluate immunogenicity will be collected at baseline and at specified time points throughout the study. The primary immunogenicity time points are 2 and/or 4 weeks after the first dose. Measurements of RSV-specific humoral immune responses will be assessed by neutralization antibody assays. ELISA and other exploratory assays to assess humoral and cellular immune responses may be performed with stored samples. Human leukocyte antigen (HLA) type may be obtained from stored samples if needed to assess HLA-class restricted cellular responses. Additional measurements of antibody, B cell and T cell responses may also be assessed from stored samples for timepoints throughout the study as exploratory evaluations. This includes tests using a number of high-throughput functional assays and high-throughput biophysical profiling tools to comprehensively profile the humoral immune response elicited by DS-Cav1 vaccination.

The immunogenicity testing will be performed at the NIAID Vaccine Immunology Program (VIP) laboratory (formerly NVITAL and VITL), in research laboratories at the VRC, or by other approved scientific collaborators.

2. STUDY PRODUCTS

Study products are manufactured under current Good Manufacturing Practices (cGMP) by VRC/NIAID/NIH at the VRC Pilot Plant operated under contract by the Vaccine Clinical

Materials Program, Leidos Biomedical Research, Inc., Frederick, MD. Specific manufacturing information is included on the product vial label and in the IB. Quality Assurance lot release testing by the manufacturer and ongoing stability programs verify conformance to product specifications throughout use in clinical trials.

2.1. VRC-RSVRGP084-00-VP

VRC-RSVRGP084-00-VP (DS-Cav1) vaccine is a sterile, aqueous, buffered solution filled into single dose vials. Details on VRC-RSVRGP084-00-VP composition and manufacturing are found in the IB.

2.2. Aluminum Hydroxide Suspension - Adjuvant

Aluminum Hydroxide Suspension (adjuvant) is a sterile, pyrogen-free, suspension filled into glass vials at a nominal fill volume of 0.7 mL ± 0.10 mL to allow withdrawal of 0.5 mL. Aluminum concentration is 5 ± 1 mg/mL. Adjuvant is stored at 2-8°C, do not freeze.

2.3. Preclinical Toxicology Studies of VRC-RSVRGP084-00-VP

Details on preclinical studies conducted with VRC-RSVRGP084-00-VP along or with adjuvant can be found in the IB.

3. STUDY OBJECTIVES

3.1. Primary Objectives

- To evaluate the safety and tolerability of DS-Cav1 alone or with adjuvant when administered IM at a dose of 50 mcg to healthy adults.
- To evaluate the safety and tolerability of DS-Cav1 alone or with adjuvant when administered IM at a dose of 150 mcg to healthy adults.
- To evaluate the safety and tolerability of DS-Cav1 alone or with adjuvant when administered IM at a dose of 500 mcg to healthy adults.

3.2. Secondary Objectives

- To evaluate the antibody response as measured by neutralization assay of DS-Cav1 alone or with adjuvant at 2 and/or 4 weeks post first injection for each group.
- To evaluate the antibody response as measured by neutralization assay of DS-Cav1 alone or with adjuvant at 2 and/or 4 weeks post second injection for each group.

3.3. Exploratory Objectives

- To evaluate the frequency and magnitude of RSV-specific T cell, antibody and other immune responses at specified timepoints throughout the study.
- To determine if measurable levels of RSV-specific neutralizing antibodies can be found in salivary and nasal secretions at specified timepoints throughout the study.

- To evaluate epitope-specific neutralization and monoclonal antibody binding (using the Octet System, (Bio-Layer Interferometry (BLI)) by competition assays at specified timepoints throughout the study.

4. STUDY DESIGN AND CLINICAL PROCEDURES

This is an open-label, dose-escalation study to examine the safety, tolerability and dose of the DS-Cav1 vaccine alone or with adjuvant in healthy adults. A vaccine regimen of DS-Cav1 as a prime followed 12 weeks later with a DS-Cav1 boost will be compared to a vaccine regimen of an adjuvanted DS-Cav1 prime followed 12 weeks later with an adjuvanted DS-Cav1 boost for each dose level. The last 5 subjects enrolled in each group will be offered the option not to receive the boost at Week 12, such that immunogenicity of a single vaccine dose can be evaluated. The hypotheses are that the vaccine will be safe and tolerable for human administration and will induce detectable immune responses. The primary objective is to evaluate the safety and tolerability in healthy adults of the investigational vaccine alone and with adjuvant. Secondary objectives are related to the immunogenicity of the vaccine alone and with adjuvant. The study schema is shown in Table 1.

Table 1: VRC 317 Study Schema

VRC 317 Study Schema				
Group	**Subjects**	**Dose**	**Day 0**	**Week 12[1]**
1	15	50 mcg	DS-Cav1	DS-Cav1
2	15		DS-Cav1 + alum	DS-Cav1 + alum
3	15	150 mcg	DS-Cav1	DS-Cav1
4	15		DS-Cav1 + alum	DS-Cav1 + alum
5	15	500 mcg	DS-Cav1	DS-Cav1
6	15		DS-Cav1 + alum	DS-Cav1 + alum
Total	90*	All DS-Cav1 vaccinations are administered with needle and syringe into the deltoid muscle. *Up to 100 subjects may be enrolled if needed to evaluate safety or immunogenicity. [1] This dose is optional for the last 5 subjects enrolled in each group that receive the Day 0 injection and any additional subjects enrolled to evaluate safety or immunogenicity.		

The study will be conducted at the VRC Vaccine Evaluation Clinic (VEC) in the NIH Clinical Center (NIH CC). The visit schedule is shown in Appendix III. The expected duration of time on study per subject is approximately 44 weeks.

4.1. Eligibility Criteria

All inclusion and exclusion criteria must be met for eligibility.

4.1.1. Inclusion Criteria

A subject must meet all of the following criteria:

1. 18 to 50 years of age.
2. Willing and able to complete the informed consent process.
3. Available for clinic visits through 44 weeks after enrollment.
4. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
5. Willing to donate blood and mucosal samples to be stored and used for future research.
6. In good general health without clinically significant medical history.
7. Physical examination and laboratory results without clinically significant findings and a Body Mass Index (BMI) ≤40 within the 56 days prior to enrollment.

*Laboratory criteria within **56 days prior to enrollment:***

8. WBC and differential either within institutional normal range or accompanied by Principal Investigator (PI) or designee approval.
9. Platelets = 125,000 – 500,000/mm3.
10. Hemoglobin within institutional normal range.
11. Creatinine ≤ 1.1 x ULN.
12. ALT ≤1.25 x ULN.
13. Negative for HIV infection by an FDA approved method of detection.

Criteria applicable to women of childbearing potential:

14. Negative result on a human chorionic gonadotropin pregnancy test on day of enrollment before receiving study product.
15. Agree to use effective means of birth control from at least 21 days before enrollment through 4 weeks after the last injection.

4.1.2. Exclusion Criteria

A subject will be excluded if one or more of the following conditions apply:

Criteria applicable to women of childbearing potential:

1. Breast-feeding or planning to become pregnant through 4 weeks after the last injection.

Subject has received any of the following:

2. More than 10 days of systemic immunosuppressive medications or cytotoxic medications within the 4 weeks prior to enrollment or any within the 14 days prior to enrollment.

3. Blood products within 16 weeks prior to enrollment.
4. Live attenuated vaccines within 4 weeks prior to enrollment.
5. Inactivated vaccines within 2 weeks prior to enrollment.
6. Investigational research agents within 4 weeks prior to enrollment or planning to receive investigational products while on the study.
7. Current allergen immunotherapy with antigen injections, unless on maintenance schedule.
8. Current anti-TB prophylaxis or therapy.

Subject has any of the following:

9. Serious reactions to vaccines that preclude receipt of study injections as determined by the investigator.
10. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema.
11. Asthma that is not well controlled.
12. Diabetes mellitus (type I or II), with the exception of gestational diabetes.
13. Thyroid disease that is not well controlled.
14. Hypertension that is not well controlled.
15. Evidence of autoimmune disease or immunodeficiency.
16. Idiopathic urticaria within the past year.
17. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws.
18. Malignancy that is active or history of malignancy that is likely to recur during the study.
19. Seizure disorder other than: 1) febrile seizures, 2) seizures secondary to alcohol withdrawal more than 3 years ago, or 3) seizures that have not required treatment within the last 3 years.
20. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen.
21. Psychiatric condition that precludes compliance with the protocol; past or present psychoses; or within 5 years prior to enrollment, a history of suicide plan or attempt.
22. Any medical, psychiatric, or social condition that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a subject's ability to give informed consent.

4.2. Clinical Procedures and Evaluations

Evaluation of this investigational vaccine will include laboratory tests, medical history, physical assessment by clinicians and subject self-assessment. The schedule of study visits is shown in

the Schedule of Evaluations (Appendix III). Total blood volume drawn from each subject will not exceed 550 mL in any 8 week period.

4.2.1. Screening

Screening for this study will be completed through the VRC's screening protocol, VRC 500 (NIH 11-I-0164). Subjects will be recruited through Institutional Review Board (IRB)-approved advertising. Evaluations and sample collections for screening processes include a medical history review, physical exam, any laboratory tests needed to confirm eligibility, and pregnancy test for women of reproductive potential. If screening evaluations suggest a concerning health condition, appropriate evaluations should be conducted based on clinical judgment. Screening evaluations for specific eligibility criteria must be completed within the time interval specified prior to enrollment for the given parameter and may be repeated, as needed, to confirm eligibility. Blood samples for research must be collected during screening; although generally collected in the 56 days prior to enrollment, a particular interval of time prior to enrollment for collection of these samples is not specified.

The informed consent form (ICF) will be reviewed and counseling related to pregnancy prevention will be provided. Subjects who are not up to date on standard vaccinations may receive these, if available, during screening or at a later date during study participation. As part of the informed consent process, an Assessment of Understanding (AoU) is completed on the day the subject is scheduled to enroll, prior to signing the VRC 317 ICF. Incorrect answers will be explained by the study clinician, and the VRC 317 ICF may be signed once the clinician is satisfied with the subject's understanding of the study.

4.2.2. Enrollment

Day 0 is defined as the day of protocol enrollment and first study injection. Protocol-specific eligibility is reviewed on Day 0 as part of the enrollment process, but eligibility evaluations conducted during a screening visit are routinely used for eligibility if the screening occurred within the specified window prior to the Day 0 visit. However, if clinical assessment on Day 0 suggests significant changes may have occurred since the screening visit, then evaluations done on Day 0 are used for eligibility. Day 0 evaluations and medical history prior to the first injection are the baseline for subsequent safety assessments.

4.2.3. Product Administration

On each injection day (prior to injection), laboratory and research samples will be collected and subjects will be clinically evaluated. A subject who arrives at the clinic with fever or evidence of an acute illness, which precludes administration of the vaccine, may be rescheduled within the allowed visit window. Pregnancy test results for females of reproductive potential must be confirmed as negative within 24 hours before each injection.

When choosing a site for injection, clinicians should consider whether there is an arm injury, local skin problem or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection. It is recommended, but not required, that the first injection be administered into the non-dominant arm. It is preferred, but not required, to alternate arms for study injections.

All injections will be administered IM into upper arm deltoid muscle by needle and syringe. For groups receiving adjuvant, the adjuvant will be field mixed by the site pharmacist prior to administration.

4.2.4. Post-Injection Follow-Up and Diary Card

Post-Injection Follow-Up: Following each study injection, subjects will be observed for a minimum of 60 minutes. Vital signs (temperature, blood pressure and pulse) will be taken at least 60 minutes post-injection. The injection site will be inspected for evidence of local reaction. In keeping with the institution's policy and good medical practice, acute medical care will be provided to subjects for any immediate allergic reactions or other injury resulting from participation in this research study.

Study clinicians must inform subjects to contact the clinic at any time after each study injection if the subject has any concerning signs or symptoms. Follow-up on subject well-being will be performed by telephone on the first or second day following all injections and by clinic visits as shown in the Schedule of Evaluations (Appendix III).

Events following any study injection that may require clinical evaluation include rash, urticaria, fever of 38.5˚C (Grade 2) or higher lasting greater than 24 hours, or significant impairment in the activities of daily living. Other clinical concerns may prompt a study visit based on clinical judgment.

Oral and nasal mucosal samples will be collected at specified study visits according to the Schedule of Evaluations in Appendix III. A small ophthalmic sponge designed for clinical use will be used to collect oral mucosal samples. Flocked swabs will be used to collect the nasal mucosal samples. These samples are for research purposes only and are not used for evaluating subject health.

Diary Card: Subjects will be given a "Diary Card" to use as a memory aid, on which to record temperature and symptoms daily for 7 days after each injection. Subjects will be trained and encouraged to use the secure electronic database, but will also have the option to complete a paper diary card. When the diary card parameters are recorded directly by the subject in the electronic database, the subject's electronic record will be the source for these data. When collected, the paper diary card will be used as a source document. When neither a paper nor electronic diary is available from the subject, the clinician will document the source of reactogenicity information recorded in the study database.

The solicited signs and symptoms on the diary card will include the following parameters: unusually tired/feeling unwell, muscles aches (other than at injection site), headache, chills, nausea, and pain/tenderness at injection site. Subjects will also record the day's highest measured temperature and measurement of largest diameter for redness and swelling at injection site.

4.2.5. Follow-Up through End of Study

Study follow-up will continue via clinical visits through 44 weeks after the first study injection. The visit schedule is based on intervals of time after each study injection administration. Any subject who receives at least one study injection (including those subjects who elect not to

receive the optional second dose) will be expected to continue with follow-up for safety and immunogenicity through study Week 44 as per Schedule of Evaluations (Appendix III).

Throughout the study, any subject who is suspected of having an upper respiratory infection based on the presence of rhinorrhea in conjunction with at least one additional sign and/or symptom should be evaluated by nasal flock swab for PCR diagnostic purposes.

The schedule of visits, allowable windows for completing the visits, and evaluations performed at each visit are shown in the Schedule of Evaluations in Appendix III. After Day 0, deviations from the visit windows in completing study visits are discouraged and will be recorded as protocol deviations, but are permitted at the discretion of the PI.

4.2.6. Concomitant Medications

Only routine prescription medications at the time of enrollment are recorded in the study database. Subsequently, concomitant medications are only updated or recorded in the study database if there is an occurrence of an adverse event (AE) that requires expedited reporting or if the subject develops a new chronic condition that requires ongoing medical management. Receipt of a FDA-approved vaccine at any time during the study will be recorded in the database (clinicians should work with subjects regarding the timing of licensed vaccines relative to study injection). Otherwise, the concomitant medication changes throughout the study will be recorded in the subject's chart as needed for general medical records, but will not be recorded in the study database.

4.3. Dose Continuation and Dose Escalation Plans

Dose groups will be enrolled sequentially, with no more than one subject per day for the first 2 study injections of the study. Dose continuation and escalation will occur after subjects have completed the first injection and following interim safety data reviews. Approval to proceed will be obtained from the Protocol Safety Review Team (PSRT).

- The dose continuation review (for 50 mcg) will occur when at least 5 subjects who received 50 mcg in either Group 1 or Group 2 have completed post-injection follow-up through completion of the 7 day diary card. If assessed as safe to continue, enrollment of additional subjects in Group 1 and Group 2 may proceed.
- The first dose escalation review (50 mcg to 150 mcg) will occur when at least 10 subjects who received 50 mcg in either Group 1 or Group 2 have completed post-injection follow-up through at least the "Study Week 2" visit. If assessed as safe, enrollment of additional subjects may proceed until at least 10 subjects have enrolled in each Group 1 and Group 2. After that, enrollments may begin in Group 3 and Group 4.
- The second dose escalation review (150 mcg to 500 mcg) will occur when at least 10 subjects who received 150 mcg in either Group 3 or Group 4 have completed post injection follow-up through at least the "Study Week 2" visit. If assessed as safe, enrollment of additional subjects may proceed until at least 10 subjects have enrolled in each Group 3 and Group 4. After that enrollments may begin in Group 5 and Group 6.

- The final dose continuation review (for 500 mcg) will occur when at least 10 subjects who received 500 mcg in either Group 5 or Group 6 have completed post injection follow-up through at least the "Study Week 2" visit. If assessed as safe, enrollment of additional subjects may proceed until at least 10 subjects have enrolled in each Group 5 and Group 6.
- The second injection for all groups may occur once the first dose of that dose level has been assessed as safe at the "Study Week 2" visit.

If the first study injection is not completed or there are discontinuations from the study before there are sufficient data to conduct the dose escalation review for a group, then extra subjects may be enrolled into that group in order to have the requisite data on at least 10 subjects. Additionally, AEs assessed as related to the study product at the time of a dose escalation review may be judged by the PSRT to warrant adding additional subjects at a given dose level.

The IRB will be provided with documentation of the safety review process and notification of the dose escalation. Consultation with the IRB and FDA, if needed, as per study pause criteria (Section 4.4) will occur if indicated by the review. One outcome of a dose escalation review may be to recommend evaluation of additional subjects at the current dose level and reassess for safety before proceeding to a higher dose level.

4.4. Criteria for Discontinuing Subject Participation

Decisions to discontinue study injections or protocol participation for a subject will be made by the PI.

4.4.1. Discontinuation from Study Injections

A subject may be discontinued from study injections for the following reasons:

- Pregnancy;
- Grade 3 AE assessed as related to study product (except that self-limited Grade 3 solicited reactogenicity does not require discontinuation of injections);
- Grade 4 AE assessed as related to study product;
- Immediate hypersensitivity reaction associated with study product;
- Intercurrent illness that is not expected to resolve before the next scheduled injection;
- Treatment with systemic glucocorticoids (e.g., prednisone or other glucocorticoid) or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that study injection may continue per PI discretion if the next one occurs at least 2 weeks following completion of glucocorticoid treatment; or,
- The PI assesses that it is not in the best interest of the subject to continue receiving study product.

4.4.2. Discontinuation from Protocol Participation

A subject may be discontinued from protocol participation for the following reasons:

- Subject voluntarily withdraws;
- The IND Sponsor or regulatory authorities stop the study; or,
- The PI assesses that it is not in the best interest of the subject to continue participation in the study or that the subject's compliance with the study is not sufficient.

4.5. Criteria for Pausing and Resuming the Study

4.5.1. Criteria for Pausing the Study

The PI and PSRT will closely monitor and analyze study data as they become available and will make determinations regarding the presence and severity of AEs. Enrollments and administration of study product will be paused by the PI, and the IND Sponsor will be promptly notified, according to the following criteria:

- **One** (or more) subject experiences a **Serious Adverse Event (SAE)** assessed as related to study product.
- **Two** (or more) subjects experience the same **Grade 3 or higher unsolicited AE** assessed as related to the study product.

4.5.2. Plan for Review of Pauses and Resuming Rules

The IND Sponsor with participation by the PSRT, will conduct the review and make the decision to resume, amend or close the study. As part of the pause review, the reviewers will also advise on whether the study needs to be paused again for any subsequent AEs of the same type.

Enrollments and study product administration would resume only if review of the AEs that caused the pause result in a recommendation to permit further study injections and enrollments. When indicated, safety data reports and changes in study status will be submitted to relevant regulatory authorities including the IRB in accordance with Section 5.3 and institutional policy.

5. SAFETY AND ADVERSE EVENTS

5.1. Adverse Events

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the use of study treatment, whether or not considered related to the study treatment.

AEs will be graded according to the Table for Grading Severity of AEs (Appendix IV). The following guidelines will be used to determine if an AE should be recorded in the database:

- Solicited AEs (i.e. reactogenicity parameters) will be recorded in the study database for 7 days after each study injection without the collection of product attribution assessments.

Clinicians will follow and collect resolution information for any reactogenicity symptoms that are not resolved within 7 days.

- Unsolicited AEs will be recorded in the study database from the date of receipt of each study injection through the visit scheduled at 28 days after each injection with the collection of product attribution assessments. At other time periods between injections and when greater than 28 days after the last injection, only SAEs (as detailed in Section 5.2) and new chronic medical conditions will be recorded as an AE through the last study visit.

5.2. Serious Adverse Events

5.2.1. Serious Adverse Event Definition

The term "serious adverse event" (SAE) as defined in 21 Code of Federal Regulations (CFR) 312.32 as follows: "an adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

"Life threatening" refers to an AE that at occurrence represented an immediate risk of death to the subject. An event that hypothetically may cause death if it occurs in a more severe form is not considered life-threatening. Similarly, a hospital admission for an elective procedure is not considered a SAE.

5.2.2. Reporting Serious Adverse Events to the IND Sponsor

AEs that meet SAE Reporting Requirements must be reported by the clinical site and submitted on an expedited basis to the IND Sponsor, according to sponsor guidelines as follows:

- results in death;
- is life-threatening;
- results in persistent or significant disability/incapacity;
- requires unplanned inpatient hospitalization or prolongation of existing hospitalization;
- is a congenital anomaly/birth defect in the offspring of a study subject; or,
- is an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Additionally, any event, regardless of severity, which in the judgment of a PI represents a SAE, may be reported on an expedited basis.

An investigator will communicate an initial SAE report within 24 hours of site awareness of occurrence to the IND Sponsor by email to the VRC Protocol Operations Office (Appendix II). A written report by the investigator should be submitted to the IND Sponsor within 3 working days. In order for the IND Sponsor to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 or 15 calendar days, the investigator must submit additional information as soon as it is available.

5.2.3. IND Sponsor Reporting to the FDA

It is the responsibility of the IND Sponsor to make the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs) as defined in 21 CFR 312.32.

- *Suspected adverse reaction* means any AE for which there is a reasonable possibility that the drug caused the AE.
- *Unexpected adverse event* means an AE that is not listed in the IB or is not listed at the specificity or severity that has been observed.

All SUSARs, as determined by the IND Sponsor, will be reported to the FDA as IND Safety Reports. All IND Safety Reports will be provided to the IRB.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

5.3. Reporting to the Institutional Review Board

The following information is consistent with NIH IRB Policy 801: Reporting Research Events, Version 1, effective July 1, 2019.

Reportable Event - An event that occurs during the course of human subject research that requires notification to the IRB.

For the purposes of this policy, reportable events include the following:

- Unanticipated Problems (UPs) involving risks to subjects or others,
- Non-compliance (including major protocol deviations and noncompliance that is not related to a protocol deviation),
- Deaths related or possibly related to research activities, and
- New information that might affect the willingness of subjects to enroll or continue participation in the study.

5.3.1. Unanticipated Problem

An unanticipated problem (UP) is defined as any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB

approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; **and**

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); **and**
- Suggests that the research places subjects or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

5.3.2. Non-Compliance

Non-compliance is the failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

Non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

Non-compliance is further characterized as serious or continuing as follows:

- Serious non-compliance - Non-compliance, hether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
- Continuing non-compliance – A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events.

Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported to the IRB by the PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware.

5.3.3. Protocol Deviation

A protocol deviation is defined as any change, divergence, or departure from the IRB-approved research protocol and is further characterized as major and minor as follows:

- Major Deviations – Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.

- Minor Deviations – Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

For the reporting purposes, failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject.

A major deviation must be reported within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although PDs are also non-compliance, these should only be reported once as deviations. Major deviations resulting in death must be reported within 24 hours of the occurrence of the event or of any member of the study team becoming aware of the death.

Researchers are responsible for monitoring their studies throughout the year for adherence to the IRB-approved protocol. The purpose of this monitoring is to identify major deviations and to look for trends in minor deviations that may indicate a systemic issue in how the study is being conducted that could potentially negatively impact the rights, safety, or welfare of participants or the study's ability to produce scientifically valid results. A series of minor deviations pointing toward a more global issue that could affect the rights, safety or welfare of the participant or affect the validity of the study should be reported as a major deviation. In all other instances, a summary of minor deviations should be provided to the IRB at the time of continuing review.

5.3.4. Death

Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

5.3.5. New Information

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

5.3.6. Suspension or Termination of Research Activities

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency must be reported within 7 calendar days of an investigator becoming aware.

5.3.7. Expedited Reporting to the IRB

Death related to research must be reported within **24 hours**.

The following will be reported within **7 calendar days** of investigator awareness:

- Actual or suspected UPs;
- Actual or suspected non-compliance;
- Actual or suspected Major PDs;
- SAEs that are actual or suspected UPs;

- New information that might affect the willingness of a subject to enroll or remain in the study;
- Suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency..

5.3.8. Annual Reporting to the NIAID IRB

The following will be reported to the IRB in summary at the time of Continuing Review:

- Summary of UPs and non-compliance;
- AEs, including SAEs, that are not UPs, as a narrative summary statement indicating whether these events were within the expected range;
- Minor PDs (aggregate summary);
- Any trends or events which in the opinion of the investigator should be reported.

6. STATISTICAL CONSIDERATIONS AND SAMPLE ANALYSIS

6.1. Overview

This is a dose-escalation study to evaluate the safety and immunogenicity of a 2-dose regimen with the DS-Cav1 vaccine given alone or with adjuvant. As available, the effect of a 1-dose regimen will also be evaluated in up to 5 subjects in each group who will be offered the option to receive only a single administration of the study product. The primary objectives relate to safety and tolerability; the secondary and exploratory objectives relate to immunogenicity and durability of immune responses.

6.2. Endpoints

6.2.1. Primary Endpoints: Safety

Assessment of product safety will include clinical observation and monitoring of hematological and chemical parameters. Reactogenicity will be closely monitored for 7 days after each product administration and safety evaluated by clinical visits through the study duration.

The following parameters will be assessed:

- Local reactogenicity signs and symptoms
- Systemic reactogenicity signs and symptoms
- Laboratory measures of safety
- Adverse events
- Serious adverse events
- New chronic medical conditions

6.2.2. Secondary Endpoints: Immunogenicity

The principal immunogenicity endpoints are antigen-specific antibody responses as evaluated by neutralization assays. The primary timepoints for immunogenicity evaluation are 2 and/or 4 weeks after the first injection for each group. Immunogenicity after the second injection will also be evaluated when applicable.

6.2.3. Exploratory Endpoints

ELISA, neutralization assay, and T cell assays performed with research samples collected at study timepoints as well as other immunogenicity assays throughout the study may be completed as exploratory evaluations.

6.3. Sample Size and Accrual

The study design is to have 90 healthy adult subjects divided equally among 6 groups.

6.3.1. Power Calculations for Evaluation of Safety

The goal of the safety evaluation for this study is to identify safety concerns associated with injections of the investigational vaccine. Primary sample size calculations for safety are expressed in terms of the ability to detect serious adverse experiences**.**

Table 2 describes the probability of observing 0 and 2 or more adverse events among 10, 15, 30, and 90 subjects, based on a range of true underlying probabilities. For example, if an adverse event of interest has a true incidence rate of 1%, there is an 86% chance that we won't see any events in a group of 15 participants. However, if this same 1% incidence applies to all 90 participants in the study, there is a 60% (100-40%) chance of observing at least one occurrence and a 23% chance of observing 2 or more.

Table 2: Probability of Observing Adverse Events

Probability of Adverse Event	Prob. 0/10	Prob. 2+/10	Prob. 0/15	Prob. 2+/15	Prob. 0/30	Prob. 2+/30	Prob. 0/90	Prob. 2+/90
0.01	0.90	<0.01	0.86	0.01	0.74	0.04	0.40	0.23
0.05	0.60	0.09	0.46	0.17	0.21	0.45	0.01	0.94
0.10	0.35	0.26	0.21	0.45	0.04	0.82	<0.01	>0.99

If we do not observe any AEs in a group of 10 (for example, at the dose escalation review), the upper 95% confidence interval for the true incidence rate at that dose is 31%; that is, we can be reasonably confident that the true rate is no more than 31%. If we do not observe any of a specific type of adverse events in a specific arm (n=15), the upper bound of a 95% confidence interval for the true incidence is 21.8%. Similarly, if we do not observe any of a specific type of

adverse events in a pair of arms (such as 1 & 2; n=30), the upper bound of a 95% confidence interval for the true incidence is 12%.

6.3.2. Power Calculations for Evaluation of Immunogenicity

Although immunogenicity is a secondary endpoint, it is of sufficient interest that we briefly present the power to detect a difference between immune responses before and after the first dose, and between two dose cohorts. Based on previous research[44], we expect the ELISA and neutralization results to be approximately normally distributed on the $\log_{10}$ scale, with standard deviation approximately 0.2-0.5 $\log_{10}$. Using the conservative value of 0.5$\log_{10}$ for the true standard deviation, we will have good power (90% or better) to detect a difference in the magnitude of the immune response of approximately .45 $\log_{10}$ with 15 per group. With 30 per group, we will be able to detect a difference in the average magnitude of approximately 0.3 $\log_{10}$.

6.4. Statistical Analysis

Since enrollment is concurrent with receiving the first study vaccination, the expectation is that all participants will receive at least one vaccination and therefore will provide some safety data. All statistical analyses will be performed using statistical software R. No formal multiple comparison adjustments will be employed for safety endpoints or immunogenicity endpoints.

6.4.1. Baseline Demographics

Baseline characteristics including demographics and laboratory measurements will be summarized using descriptive statistics.

6.4.2. Safety Analysis

Reactogenicity: The number and percentage of subjects experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each subject's reactogenicity will be counted once under the maximum severity for all assessments.

Adverse Events: AEs will be coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentage of subjects experiencing each specific AE will be tabulated by severity and relationship to study product. For the calculations in these tables, each subject's AE will be counted once under the maximum severity or strongest recorded causal relationship to treatment.

A complete listing of AEs for each subject will provide details including severity, relationship to treatment, onset, duration and outcome.

Safety Laboratory Values: Boxplots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

6.4.3. Analysis of Immune Responses

The primary immunogenicity time points are 2 and/or 4 weeks after the first dose, although immunogenicity data at other time points will be examined as well. The magnitude of the

responses will be compared within subjects (before and after first dose; before and after second dose) and between subjects (between dose cohorts and between those who receive Alum and those who do not). The primary comparisons at 2 and/or 4 weeks post first dose will be compared using Wilcoxon Rank Sum tests, but regression models will also be explored that model post-dose immune response as a function of pre-dose immune response, dose, Alum (yes/no), and possibly demographic variables such as age and gender. Immunogenicity will be analyzed for a variety of cohorts defined based on vaccination status, and specified in the Statistical Analysis Plan (SAP). At minimum these may include ITT, PP, and mITT cohorts.

6.4.4. Missing Data

Missing responses will be assumed to be missing completely at random. Analyses will include all samples available at each study time point.

6.4.5. Interim Analyses

An interim analysis of immunogenicity data may be performed when at least 10 subjects from Groups 1-4 reach 4 weeks post first dose. Reports will be provided to the PI and other key VRC investigators for the purpose of informing future trial-related decisions in a timely manner and may be published as preliminary data. Results should in no way influence the conduct of the trial in terms of early termination or later safety or immunogenicity endpoint assessments.

6.4.6. Randomization of Treatment Assignments

At the start of the study, subjects will be randomized 1:1 to Groups 1 and 2. If the criteria for the first Dose Escalation are met, procedures for the interim safety review are completed, and there are at least 10 people enrolled in each of Groups 1 and 2, subjects will be randomized 1:1 in Groups 3 and 4. If the criteria for the second Dose Escalation are met, procedures for the interim safety review are completed, and there are at least 10 people enrolled in each of Groups 3 and 4, subjects will be randomized 1:1 in Groups 5 and 6. Once all groups have at least 10 subjects per group, or if the dose escalation criteria are not met, subjects will be randomized in equal proportions to all groups that are open.

The subject and the study clinicians will be informed of the subject's group assignment upon completing the enrollment in the database.

To decrease the potential for participant dropouts during the period between randomization and initial vaccination, randomization will occur on Day 0 after the study consent is signed and eligibility is confirmed. If subjects accrued to a study group do not complete the number of injections and/or a follow-up duration specified, then additional subjects may be accrued in that group.

7. PHARMACY PROCEDURES

7.1. Study Products

The study products are prepared under cGMP by the VRC Pilot Plant and must meet lot release specifications prior to clinical use. This study includes one investigational vaccine, one adjuvant, and one diluent as follows:

- VRC-RSVRGP084-00-VP (DS-Cav1) drug product is a sterile, aqueous, buffered solution filled under aseptic conditions into single dose vials at a concentration of 500 mcg/mL. The formulation buffer is comprised of 20mM Histidine (buffer), 100mM Potassium Chloride (isotonic acid), 100mM Arginine HCl (stabilizer) and 2.5% weight per volume Sucrose (stabilizer) at pH 6.5. The drug product is aseptically filled at a volume of 1.2 ± 0.10 mL in a 3 mL glass vial. Vials contain a clear to slightly hazy sterile solution; some small white translucent particles may be visible.
- Aluminum Hydroxide Suspension, alum, adjuvant is composed of Alhydrogel® 2% (Brenntag Biosector, Frederikssund Denmark) diluted with water for injection to a concentration of 5 mg/mL. Adjuvant is aseptically filled at a volume of 0.7 ± 0.1 mL.
- VRC-PBSPLA043-00-VP, diluent, is comprised of Phosphate Buffered Saline (PBS) aseptically filled into single dose vials at a volume of 1.2 ± 0.10 mL.

7.2. Study Product Presentation and Storage

7.2.1. Labels

At the time of study product delivery to the pharmacy, study products labels will have specific product information (e.g., lot number, fill volume, storage temperature). Labels will contain an Investigational Use Statement ("Limited by Federal Law to Investigational Use") and manufacturer information.

7.2.2. Storage

VRC-RSVRGP084-00-VP: DS-Cav1 vaccine vials will be shipped within the recommended temperature range using appropriate shipping configurations, to the study pharmacist or designee. Vials of vaccine should be stored until use at -35°C to -15ºC in a qualified, continuously monitored, non-frost-free freezer until use. As freezer temperatures may fluctuate, a temperature range of -45°C to -10°C is acceptable. Storage below -45ºC is not permitted because of the stopper limitation.

Vials, intended for single use only, do not contain preservative and should not be refrozen after thaw. Vaccine vials are removed from the freezer and equilibrated to room temperature prior to product administration.

Aluminum Hydroxide Suspension: Vials of alum are stored until use at 2ºC to 8ºC in a qualified, continuously monitored refrigerator. Do not freeze. Vials of adjuvant are intended for single use only.

VRC-PBSPLA043-00-VP: Vials of PBS are stored until use between -45°C to -10°C in a qualified, continuously monitored, non-frost-free freezer. Vials of diluent are intended for single use only and should not be refrozen after thawing.

7.2.2.1. Deviations in Temperatures

Temperature excursions that are outside of the specified ranges will be reported per pharmacy guidelines. If deviations in storage temperature occur from the normal allowance, the site pharmacist or designee must report the storage temperature excursion promptly to the PI and IND Sponsor and product must be quarantined in a separate area. The excursion must be

evaluated and investigated and action must be taken to restore and maintain the desired temperature limits. Pending the outcome of the investigation, the IND Sponsor will notify the pharmacist or designee if continued clinical use of the product is acceptable.

7.3. Preparation of Study Products for Administration

Refer to the group assignment for the study subject. The pharmacy will label the syringe before delivery to the clinic with the subject identifier, the date, and the time allowance for administration.

The following general instructions apply to all vaccine dose preparations (alone and with adjuvant) for injection.

- Preparation will be done by a Pharmacist or designee in a clean preparation unit with limited access;
- Prepare all doses under sterile conditions;
- Thaw the vial(s) containing VRC-RSVRGP084-00-VP and VRC-PBSPLA043-00-VP at ambient temperature (15ºC to 25ºC);
- The vaccine (alone and with adjuvant) is stable in the vial and syringe for 8 hours refrigerated or at room temperature following removal from recommended storage conditions;
- Administer within 8 hours following removal of vaccine from freezer;
- Keep the prepared syringe at room temperature and out of direct sunlight;
- Inversion of a prepared vial or syringe is defined as a gentle, complete 180 degree rotation of the container.

7.3.1. Preparation of VRC-RSVRGP084-00-VP (non-adjuvanted)

- For 500 mcg dose: withdraw 1.0 mL (500 mcg) into a syringe for administration.
- For 150 mcg dose:
  - Transfer 0.45 mL (225 mcg) of vaccine into a new sterile vial using a syringe,
  - Transfer 1.05 mL of PBS diluent into the same vial. (Total preparation volume = 1.5 mL),
  - Invert vial 5x to mix,
  - Withdraw 1.0 mL (150 mcg) into a new syringe for administration.
- For 50 mcg dose:
  - Transfer 0.15 mL (75 mcg) of vaccine into a new sterile vial using a syringe,
  - Transfer 1.35 mL of PBS diluent into the same vial (Total preparation volume = 1.5 mL),
  - Invert vial 5x to mix,
  - Withdraw 1.0 mL (50 mcg) into a new syringe for administration.

7.3.2. Preparation of VRC-RSVRGP084-00-VP with Adjuvant

Vials of alum and diluent will be provided for field mixing.

- For 500 mcg dose with adjuvant:
  - Add 0.12 mL of adjuvant directly to a new vial of VRC-RSVRGP084-00-VP (1.2 mL vaccine; total preparation volume = 1.32 mL),
  - Invert vial 5x to mix, incubate at ambient temperature (15ºC to 25ºC) for 15 minutes,
  - Withdraw 1.1 mL into a new syringe for administration,
  - Invert syringe 5x to mix,
  - If not administered immediately, invert syringe 5x to mix immediately prior to administration.
- For 150 mcg dose with adjuvant:
  - Transfer 0.45 mL (225 mcg) of vaccine into a new sterile vial using a syringe,
  - Transfer 0.15 mL of adjuvant into the same vial,
  - Invert vial 5x to mix, incubate at ambient temperature (15ºC - 25ºC) for 15 minutes,
  - Transfer 0.9 mL of PBS diluent into the same vial, invert 5x to mix. (Total preparation volume = 1.5 mL),
  - Withdraw 1.0 mL (150 mcg) into a new syringe for administration,
  - Invert syringe 5x to mix,
  - If not administered immediately, invert syringe 5x to mix immediately prior to administration.
- For 50 mcg dose with adjuvant:
  - Transfer 0.15 mL (75 mcg) of vaccine into a new sterile vial using a syringe,
  - Transfer 0.15 mL of adjuvant into the same vial,
  - Invert vial 5x to mix, incubate at ambient temperature (15ºC to 25ºC) for 15 minutes,
  - Transfer 1.2 mL of PBS diluent into the same vial, invert 5X to mix. (Total preparation volume = 1.5 mL),
  - Withdraw 1.0 mL (50 mcg) into a new syringe for administration,
  - Invert syringe 5x to mix,
  - If not administered immediately, invert 5x to mix immediately prior to administration.

7.4. Study Product Administration

Injections must be administered within the time allowance on the syringe label using standard needle and syringe injection technique. The study product will be administered IM into the deltoid muscle by needle and syringe.

The plan for injection administration is to use standard injection techniques as follows:

- Group 1: Administer one injection of 1.0 mL of the 50 mcg/mL preparation for each 50 mcg non-adjuvanted dose;
- Group 2: Administer one injection of 1.0 mL of the 50 mcg/mL field mixed with alum for each 50 mcg dose;
- Group 3: Administer one injection of 1.0 mL of the 150 mcg/mL preparation for each 150 mcg non-adjuvanted dose;
- Group 4: Administer one injection of 1.0 mL of the 150 mcg/mL field mixed with alum for each adjuvanted 150 mcg dose;
- Group 5: Administer one injection of 1.0 mL of the 500 mcg/mL preparation for each non-adjuvanted 500 mcg dose;
- Group 6: Administer one injection of 1.1 mL of the 500 mcg/mL field mixed with alum for each adjuvanted 500 mcg dose.

7.5. Study Product Accountability

7.5.1. Documentation

The study pharmacist or designee will be responsible for maintaining an accurate record of the codes, inventory, and an accountability record of vaccine supplies for this study. Electronic documentation as well as paper copies will be used.

7.5.2. Disposition

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved following the injection. Any unopened vials that remain at the end of the study will be discarded at the discretion of the VRC in accordance with policies that apply to investigational products. Partially used vials or expired prepared doses cannot be administered to other subjects nor used for *in vitro* experimental studies and will be discarded as indicated above.

8. HUMAN SUBJECTS PROTECTION

This research will be conducted in compliance with the protocol, Good Clinical Practices (GCP) guidance, and all applicable regulatory requirements.

8.1. Institutional Review Board

A copy of the protocol, proposed ICF, and any proposed advertising material will be submitted to the IRB for review and approval.

The PI must submit and where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the ICF. The PI will notify the IRB of deviations from the protocol and AEs.

The PI will be responsible for obtaining annual IRB approval/renewal throughout the duration of the study.

8.2. Subject Recruitment and Enrollment

All study activities will be carried out at the NIH CC. Study subjects will be recruited through on-site and off-site advertising done for the screening protocol, VRC 500 (11-I-0164). Effort will be made to include women and minorities in proportions similar to that of the community from which they are recruited.

8.2.1. Participation of Children

Children are not eligible to participate in this clinical trial because this study is designed for product evaluation in adults. If the product is assessed as safe and immunogenic, other protocols designed for children may be conducted in the future.

8.2.2. Participation of Site Employees

NIH employees and members of their immediate families may participate in this protocol. VRC will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH Information Sheet on Employee Research Participation" and a copy of the "Leave Policy for NIH Employees Participating in NIH Medical Research studies."

Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the subject's employment or work situation. The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees. The employee subject's privacy and confidentiality will be preserved in accordance with NIH CC and NIAID policies. For NIH employee subjects, consent will be obtained by an individual who is independent of the employee's team. If the individual obtaining consent is a co-worker to the subject, independent monitoring of the consent process will be included through the Bioethics Consultation Service. Study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

8.3. Informed Consent

The study ICF (provided as a separate document) describes the investigational products, the purpose, methods, anticipated benefits, and potential risks of the study. Before a subject's participation in the study, it is the investigator's responsibility to ensure that written informed consent is obtained from the subject.

The acquisition of informed consent will be documented in the subject's medical records, as required by 21 CFR 312.62, and the ICF will be signed and personally dated by the subject and by the person who conducted the informed consent discussion. The original signed ICF will be placed in the medical record and a copy will be provided to the subject.

8.4. Subject Confidentiality

The investigator must ensure that the subject's anonymity is maintained and will ensure that no information identifying the subject will be released to any unauthorized party. Subjects will not be identified in any reports of this study. All records will be kept confidential to the extent provided by federal, state and local law. Medical records will be made available for review when required by authorized agencies and regulatory authorities only under the guidelines set by the U.S. Federal Privacy Act and by relevant country-specific regulatory authorities. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform the subjects that the above named representatives will review their study-related records without violating the confidentiality of the subjects. Stored study research samples are labeled by a code that only the study team can link to the subject. The requirement to maintain subject confidentiality is included in the ICF.

8.5. Risks and Benefits

8.5.1. Risks

Risk of the DS-Cav1 vaccine: This is the first study in humans of the investigational DS-Cav1 vaccine. The risks noted are based on risks of vaccines in general.

Potential side effects resulting from intramuscular injection include stinging, arm discomfort, redness of the skin, or mild bruising at vaccine injection sites.

Subjects may exhibit general signs and symptoms associated with administration of a vaccine injection, including fever, chills, rash, aches and pains, nausea, headache, dizziness and fatigue. These side effects will be monitored, but are generally short term, mild to moderate severity and usually do not require treatment.

There may be side effects from the study products, which may be serious or life threatening, that we do not yet know about.

Risks of Aluminum Hydroxide Suspension: Aluminum is the most common adjuvant used in human vaccines licensed by the FDA [57] used in billions of individuals over decades of clinical use [51]. Side effects are generally limited to minor local reactions at the injection site [51]. Other more severe local reactions like erythema, subcutaneous nodules, contact hypersensitivity and granulomatous inflammation may occur [58].

Risks of Mucosa Sample Collection: Collection of samples by swabs and wicks rubbed over the mucosal surfaces may cause momentary discomfort and, in some cases, minor bleeding.

Risks of Blood Drawing: Blood drawing may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.

Risks during Pregnancy: We do not know the possible effects of the study vaccine on the fetus or nursing infant. Therefore, women and adolescents of reproductive potential will be tested for pregnancy prior to administration of each dose of study product. Women will be asked to notify the site immediately if they suspect or learn they are pregnant during this study. In case of pregnancy, subjects will continue to be followed for safety. The subject will be contacted about the outcome of a pregnancy that begins during the study.

Other Risks: It is possible that the standard medical tests performed as part of this research protocol will result in new diagnoses. Depending on the medical findings and consequences of being provided with the new medical information about health status, the study subject may view this aspect of study participation as either a risk or a benefit. Any such information will be shared and discussed with the subject and, if requested by the subject, may be forwarded to the subject's primary health care provider for further workup and management.

Benefits: Study subjects will not receive direct health benefit from study participation. This protocol is not designed to provide treatment for any condition. Others may benefit from knowledge gained in this study that may aid in the development of an RSV vaccine. The investigational vaccine is not expected to provide protection from RSV infection.

8.6. Plan for Use and Storage of Biological Samples

To be eligible for this protocol, subjects must be willing to allow stored specimens to be used in the future for studying infectious diseases, immune function, vaccine responses, and other medical conditions. If tests show evidence of any acute or chronic condition, subjects will be informed of the results and advised to seek appropriate medical care for the condition. In general, testing performed at a research laboratory is not for diagnostic purposes and results will not be available to the study site or study subject.

8.6.1. Use of Samples, Specimens and Data

Samples, specimens, and data collected under this protocol may be used to conduct protocol-related safety and immune response evaluations, exploratory laboratory evaluations related to the type of infection the study product was designed to prevent, exploratory laboratory evaluations related to vaccine or infectious disease research in general and for research assay validation.

Genetic testing may be performed in accordance with the genetic testing information that was included in the study ICF. HLA testing may be done in association with identifying factors linked with the immune response development or progression of infections.

8.6.2. Storage and Tracking of Blood Samples and Other Specimens

All of the stored research samples are labeled by a code that only the site can link to the subject. Samples are stored at secure facilities with limited access including VIP laboratory (formerly NVITAL, Gaithersburg, MD,) and VRC Laboratories, (Building 40, Bethesda, MD) or other approved CRO facilities. Data will be kept in password-protected computers. Only investigators or designees will have access to the samples and data. Samples will be tracked in the Laboratory Information Management System (LIMS) database or using another software designed for this purpose (e.g., Freezerworks).

8.6.3. Disposition of Samples, Specimens and Data at Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Regulatory approval through the proper human subjects protection agency will be sought prior to any sharing of samples that constitutes human subjects research. The research use of stored, unlinked or unidentified samples may be exempt from the need for IRB review and approval. When appropriate, exemption may be obtained through the proper regulatory procedures.

At the time of protocol termination, samples will remain in the VIP facility or VRC laboratories or, after IRB approval, transferred to another repository. Regulatory oversight of the stored samples and data may be transferred to a stored samples protocol as part of the IRB-approved termination plan. Data will be archived by the VRC in compliance with requirements for retention of research records, or after the IRB and the IND Sponsor approval, it may be either destroyed or transferred to another repository.

8.6.4. Loss or Destruction of Samples, Specimens or Data

The NIH Intramural Protocol Deviation definition related to loss of or destruction of samples or data will be followed. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB in accordance with institutional policies. The PI will also notify the IRB if the decision is made to destroy the remaining samples.

8.7. Compensation

Compensation for time and inconvenience of study participation will be provided to subjects in accordance with the standards for compensation of the Clinical Research Volunteer Program. The compensation per visit will be $275 for injection visits and $175 for visits that include a blood draw only. Any visit that includes mucosal sample collection will be compensated an additional $50. Compensation for any clinic visit that does not include a blood draw or mucosal sample collection will be $75 and compensation for completion of electronic diary card will be $25. The total compensation for the subject is based on the number of study clinic visits and injections completed. The approximate total compensation is $2600, depending on the study visits and injections completed.

Subjects will receive compensation about 2 weeks after each completed visit by direct deposit. Compensation may need to be reported to the Internal Revenue Service (IRS) as taxable income.

8.8. Safety Monitoring

8.8.1. Protocol Safety Review Team

Close cooperation between the designated members of the Protocol Team will occur to evaluate and respond to individual AEs in a timely manner. Study clinicians will conduct a daily safety review of any new clinical data. The PSRT includes designated team members (PI, Associate Investigators, Study Coordinators, Protocol Specialist, and other study clinicians). The PSRT will review the summary study safety data reports on a weekly basis through 4 weeks after the last subject receives the final study injection in order to be certain that the vaccine has an acceptable safety profile, and will continue to monitor the study safety data reports on at least a monthly basis through completion of the study.

9. ADMINISTRATION AND LEGAL OBLIGATIONS

9.1. Protocol Amendments and Termination

Protocol amendments must be made only with the prior approval of the IND Sponsor. Agreement from the investigator must be obtained for all protocol and ICF amendments. All amendments will also be submitted to the IRB for approval.

The VRC, NIAID, NIH, FDA and other regulatory authorities reserve the right to terminate the study. The PI will notify the IRB of the study termination in writing and provide documentation to the IND Sponsor.

9.2. Study Documentation and Study Records Retention

The PI will maintain a list of appropriately qualified persons to whom trial duties have been delegated.

Source documents are original documents, data, and records from which the subject's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence. Long-term storage of source documents may be in the form of electronic files.

The investigator and staff are responsible for maintaining a comprehensive and centralized filing system of all essential study-related documentation, suitable for inspection at any time by representatives from the IND Sponsor, VRC, NIAID, NIH, IRB, FDA, and/or applicable regulatory authorities. Elements include:

- Subject files containing completed ICF and supporting copies of source documentation, and,
- Study files containing the protocol with all amendments, the IB, and copies of all correspondence with the IRB.

In addition, all original source documentation must be maintained and readily available.

All essential documentation should be retained by the institution for the same period of time required for medical records retention. The FDA requires study records to be retained for two years after marketing approval or refusal (21 CFR 312.62). No study document should be destroyed without prior written agreement between the IND Sponsor and the PI. Should the investigator wish to assign the study records to another party or move them to another location, VRC must be notified in writing of the new responsible person and/or the new location.

9.3. Data Collection, Data Sharing, and Protocol Monitoring

9.3.1. Data Collection

Clinical research data will be collected in a secure electronic web-based clinical data management system. Extracted data without subject identifiers will be sent to the protocol statistician for statistical analysis as needed.

9.3.2. Data Sharing Plan

Data generated in this study will be shared as de-identified data in the government-funded public repository, www.ClinicalTrials.gov. Data may be shared prior to publication at approved public presentations or for collaborative development and will be shared at the time of publication and no later than 1 year after the primary completion date.

9.3.3. Source Documents

The site will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP, regulatory and institutional requirements for the protection of confidentiality of subjects. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, medical records, paper diary cards, laboratory reports, pharmacy records and other research records maintained for the clinical trial.

9.3.4. Protocol Monitoring

The IND Sponsor or their authorized representatives are responsible for ensuring integrity of study data and compliance with the protocol. The PI will allow the study monitors, the IRB, and the FDA to inspect study documents (e.g., consent forms, drug distribution forms, and case report forms) and pertinent hospital or clinic records for confirmation of the study data. Site visits by study monitors will be made to monitor the following: study operations, the quality of data collected in the research records, the accuracy and timeliness of data entered in the database, and to determine that all process and regulatory requirements are met. Study monitoring visits will occur as defined by the IND Sponsor approved monitoring plan.

9.4. Language

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are readily understood.

9.5. Policy Regarding Research-Related Injuries

The study site will provide immediate medical care for any injury resulting from participation in this research. In general, VRC, the NIH, the NIH CC, and the U.S. Government will not provide long-term medical care or financial compensation for research-related injuries.

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APPENDIX I: INFORMED CONSENT FORM

The ICF has been removed from the protocol and is (instead) provided as a separate document.

APPENDIX II: CONTACT INFORMATION

APPENDIX III: SCHEDULE OF EVALUATIONS

SCHEDULE OF EVALUATIONS FOR VRC 317																	
Visit Number		01	02	02A	02B	02C	03	04	05	06	06A	06C	07	08	09	10	11
Week of Study		-8 to 0	W0	W1	W1	W1	W2	W4	W8	W12	W13	W13	W14	W16	W24	W36	W44
Day of Study		-56 to 0	D0[5]	D1	D3	D7	D14	D28	D56	D84	D85	91	D98	D112	D168	D252	D308
Clinical Evaluations	**Tube**	**Screen**															
VRC 500 Screening Consent		X															
VRC 317 AoU and Consent			X														
Medical History		X	X		X	X	X	X	X	X		X	X	X	X	X	X
[1]Targeted Physical		X	X		X	X	X	X	X	X		X	X	X	X	X	X
[2]Vital Signs		X	X		X	X	X	X	X	X		X	X	X	X	X	X
Preg. Prevention Counseling		X	X					X		X				X			
Pregnancy Test		X	X					X		X				X			
[3]Study Injection			**X**							**X**							
Begin Diary Card			X							X							
Phone Contact (or clinic visit)				X							X						
[4]HLA typing															20		
CBC differential, platelets	EDTA	3	3			3		3		3		3		3			
Creatinine and ALT	GLT	4	4			4		4		4		4		4			
HIV antibody	SST	4															
Research Samples																	
Serum	SST	16	64			16	40	40	16	40		16	40	40	40	40	40
PBMC and plasma	EDTA	40	60		20	60	60	40	20	40		60	60	40	40	40	40
Oral secretions			X										X				
Nasal secretions			X										X				
Daily Volume (mL)		**67**	**131**	**-**	**20**	**83**	**100**	**87**	**36**	**87**	**-**	**83**	**100**	**87**	**100**	**80**	**80**
Cumulative Volume (mL)		**67**	**198**	**198**	**218**	**301**	**401**	**488**	**524**	**611**	**611**	**694**	**794**	**881**	**981**	**1061**	**1141**

[1] Targeted physical exam is performed as needed, based on subject report or indications of illness.

[2] Complete post injection evaluations (temperature, BP, pulse, respiration) at least 60 minutes after each injection

[3] The second injection is optional for the last 5 subjects enrolled in each group that receive the first injection. Subjects who receive only one injection will be expected to continue with follow-up through study Week 44.

[4] HLA type blood sample is collected only once at any timepoint and is shown at Visit 09 for convenience. If HLA type is already in the medical record, do not repeat. HLA type may also be obtained from a frozen sample.

[5] Day 0=day of enrollment and first injection. Day 0 evaluations and medical history prior to first injection are the baseline for subsequent safety assessments. If clinical assessment on Day 0 suggests changes since screening, then physical exam & laboratory tests done on Day 0 are used for eligibility and are baseline for safety assessments.

Visit windows: Visits 02A, 06A (+1 day); Visit 02B (±1 day); Visit 02C, 06C (-2 days); Visit 03, 04, 05, 07, 08 (±3 days); Visit 06 (±7 days); and Visit 09, 10, 11 (±7 days). Schedule Visits 02A-06 relative to Visit 02, Visits 06A-11 relative to Visit 06.

APPENDIX IV: ASSESSMENT OF RELATIONSHIP TO VACCINE AND ADVERSE EVENT SEVERITY GRADING

Assessment of Causality Relationship of an Adverse Event to Study Vaccine:

The relationship between an adverse event (AE) and the vaccine will be assessed by the investigator on the basis of his or her clinical judgment and the definitions below.

- **Definitely Related**. The AE and administration of study product are related in time, and a direct association can be demonstrated.
- **Probably Related**. The AE and administration of study product are reasonably related in time, and the AE is more likely explained by study product than other causes.
- **Possibly Related**. The AE and administration of study product are reasonably related in time, but the AE can be explained equally well by causes other than study product.
- **Not Related**. There is not a reasonable possibility that the AE is related to the study product.

For purposes of preparing data reports in which AE attributions are limited to "**Related**" or "**Not Related**", in this protocol, the "Definitely, Probably and Possibly" attributions will be mapped to the "Related" category. The definitions that apply when these two categories alone are used are as follows:

- **Related** – There is a reasonable possibility that the AE may be related to the study product.
- **Not Related** – There is not a reasonable possibility that the AE is related to the study product.

Grading the Severity of Adverse Events:

The FDA Guidance for Industry (September 2007): "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" is the basis for the severity grading of AEs in this protocol. Several modifications were made to the table as follows:

- "Emergency room visit" is not automatically considered a life-threatening event; these words have been removed from any "grade 4' definition where they appear in the table copied from the guidance document.
- Any laboratory value shown as a "graded" value in the table that is within the institutional normal range will not be severity graded or recorded as an AE.
- Severity grading for hemoglobin decrease on the basis of the magnitude of decrease from baseline is not applicable at the grade 1 level; only absolute hemoglobin will be used to define grade 1 decrease. Increases in hemoglobin are AEs only for values above the upper limit of normal and are graded by the systemic illness clinical criteria.
- Severity grading definition for Grade 4 local reaction to injectable product (Erythema/Redness and Induration/Swelling) included added text "requiring medical attention".
- 1 X ULN was removed from the definition for PT increase.

When not otherwise specified in the table, the following guidance will be used to assign a severity grade:

Grade 1 (Mild): No effect on activities of daily living

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

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

Grade 4 (Life-threatening): Hospitalization; immediate medical intervention or therapy required to prevent death.

Grade 5 (Death): Death is assigned a Grade 5 severity.

Only the single AE that is assessed as the primary cause of death should be assigned "Grade 5" severity.

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
FDA Guidance - 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	Hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Hospitalization
[1]Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis requiring medical attention
[2] 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 requiring medical attention

[3] Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
[4] Fever (°C) (°F)	38.0 – 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	Hospitalization for arrhythmia
[5] Bradycardia - beats per Minute	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	Hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

1. In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
2. Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
3. Subject should be at rest for all vital sign measurements.
4. Oral temperature; no recent hot or cold beverages or smoking.
5. 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	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	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	Hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	Hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	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	Hospitalization

B. Tables for Laboratory Abnormalities

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

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

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
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 Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
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	--
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 institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the 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) decrease from baseline value - gm/dL	not applicable	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) decrease from baseline value – gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	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)	1.10 – 1.20 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 institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0058 PRINCIPAL INVESTIGATOR: Grace Chen, MD, MPH

STUDY TITLE: VRC 317: A Phase I Randomized, Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability and Immunogenicity of a Stabilized Prefusion RSV F Subunit Protein Vaccine, VRC-RSVRGP084-00-VP (DS-Cav1), Alone or with Alum Adjuvant, in Healthy Adults

Continuing Review Approved by the IRB on 9/9/19
Amendment Approved by the IRB on 9/9/19 (K) Date Posted to Web: 10/9/19
Study Consent, Version 7.0

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that taking part in NIH research is entirely voluntary.

- You may decide not to take part, or you may withdraw from the study at any time. There is no penalty or loss of benefits for choosing not to take part in this study or to withdraw. However, to get care at the NIH, you must be taking part in a study or be under evaluation for study participation.
- You may get no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to get (like blood transfusions). If you have these beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

PURPOSE OF THIS STUDY

This is a research study of an experimental vaccine against respiratory syncytial virus (RSV). "Experimental" means that the study vaccine has not been approved by the U.S. Food and Drug Administration (FDA). The FDA allows this vaccine to be used for research purposes only. This vaccine has never been given to humans before this study. We do not know if the vaccine works. The main purpose of this study is to see if the experimental vaccine is safe and if there are any side effects. We also want to study immune responses to the vaccine including cells that may recognize and fight RSV.

BACKGROUND ON RSV

RSV is a respiratory virus that infects the lungs and breathing passages. Healthy people who get RSV usually experience mild cold symptoms that last for one or two weeks. RSV infection can also be serious, especially for infants and older adults. RSV is the most common cause of bronchiolitis (inflammation of the small airways in the lung) and pneumonia in

children younger than 1 year of age in the U.S. Almost all children will be infected with RSV by their second birthday. RSV is also a significant cause of respiratory illness in older adults.

RSV can be spread when an infected person coughs or sneezes, creating droplets that carry the virus in the air. Others can get infected if the droplets get in their nose, mouth, or eye.

Infection can also happen from direct and indirect contact with nose or oral fluids from infected people. Direct contact with the virus can happen, for example, by kissing the face of a child with RSV. Indirect contact can happen if the virus gets on an environmental surface, like a doorknob, that is then touched by other people.

There is currently no vaccine to prevent RSV infection and no cure for RSV infection.

One experimental RSV vaccine (FI-RSV) was tested in the 1960s in babies. FI-RSV was made using whole virus that was killed by a chemical treatment. It did not prevent RSV infection in babies and it made their RSV illness worse. The babies that got FI-RSV had never had an RSV infection before. This may have played a part in their weak immune response. F1-RSV was very different from the vaccine in this study. Our vaccine has only one viral protein. This protein caused a strong immune response in animal studies. Also, all adults have had RSV infection at least once, and maybe many times, so we expect a better and stronger immune response in adults.

There is one medicine (palivizumab) that helps prevent severe RSV illness in some infants and children. But the medicine cannot help cure or treat children who already have serious RSV disease and it cannot prevent infection with RSV.

We will tell you if we learn anything new during this study that might cause you to change your mind about staying in the study. At the end of the study, we will tell you when study results may be available and how to learn about them.

STUDY PRODUCTS

Vaccines are substances that are given to help the body fight off an infection. When you get a dose of this vaccine, it will look like a protein that is on the outside surface of RSV. Your body may make an immune response based on this protein.

In this study, the vaccine is called 'VRC-RSVRGP084-00-VP' or 'DS-Cav1' or simply 'RSV vaccine'. The vaccine was developed by the Vaccine Research Center (VRC) at the NIH. It was made at the VRC Pilot Plant in Frederick, MD. This vaccine does not have any live or killed RSV in it. It is impossible for you to get RSV from this vaccine. This is the first study to give this RSV vaccine to humans. You should not expect this experimental vaccine to protect you from RSV infection.

Some people in this study will get the RSV vaccine mixed with another study product called an adjuvant. Adjuvants are substances that may make your body's response to the vaccine better. The adjuvant in this study will be aluminum hydroxide (alum). Alum has been used for over 60 years in billions of vaccinations with licensed vaccines and has been found to be safe. The use of the alum as an adjuvant in this study has been reviewed and approved by the FDA.

ELIGIBILITY

You may qualify to take part in this study if:

- you are between 18 and 50 years of age,
- you agree not to become pregnant for at least 4 weeks after you get the last injection, which is about 4 months after the study begins
- you have physical exam and blood test results that meet study requirements, and
- you do not have any serious medical problems as determined by your screening.

STUDY PLAN

The study plan includes about 90 people. Study participation will last for about 1 year with at least 13 clinic visits during that time.

The study will have 6 different groups with about 15 people in each. We will start by enrolling people to get the lowest dose of 50 mcg. We will review the data from about 10 people after 2 weeks to make sure the vaccine is safe before we start enrolling people to get the next dose of 150 mcg. We will review the data from about 10 people for this dose after 2 weeks to make sure the vaccine is safe before we start enrolling the highest dose of 500 mcg. We will continue to enroll subjects until there are about 15 people in each treatment group.

For each dose, people will be randomly assigned (like pulling a number from a hat) to get either vaccine or vaccine + alum. Once enrolled, you will know which group you are in. The 6 possible groups are shown below:

Group	Subjects	Dose	Day 0	Week 12[1]
1	15	50 mcg	vaccine	vaccine
2	15		vaccine + alum	vaccine + alum
3	15	150 mcg	vaccine	vaccine
4	15		vaccine + alum	vaccine + alum
5	15	500 mcg	vaccine	vaccine
6	15		vaccine + alum	vaccine + alum
Total	90[2]	[1] The last 5 subjects in each group that get the first injection will be allowed to choose whether or not to get the second injection at Week 12. [2] Extra subjects (up to 10 in total) may be added to any group to look at the body's response to the vaccine dose given. They will also have the choice to get the second injection at Week 12 or not.		

STUDY PROCEDURES

If you agree to take part in this study, you will get no more than two injections: the first on Day 0 and the second at Week 12. All injections will be given in the upper arm muscle using a needle and syringe. You will get the same product for both injections: either the RSV vaccine alone or the RSV vaccine mixed with alum depending on the group you are in. The last 5 subjects enrolled into each group that get the first injection will have the choice to not get the second injection at Week 12. The study clinicians will tell you if you are one of these 5 subjects who will be allowed to make this choice.

If you are a woman who is able to get pregnant, we will do a pregnancy test before you get each injection. The test must show that you are not pregnant to get the injection.

We will watch you for at least 60 minutes after you get each injection. We will ask you to complete a diary card for 7 days after each dose. You will get a password to a secure website to enter this data online. If you prefer, you can use a paper diary card instead. You will need to record your temperature and any side effects that you feel on the diary card. We will give you a thermometer to measure your temperature. You will also need to look at the injection site on your arm each day and record how it looks. We will also give you a measuring device (ruler) to measure any skin changes at

the injection site. The clinic staff will call you the next day after each injection visit and are available to you by phone 24 hours a day for you to report any concerning side effects. We will ask you to review your diary card with us.

If you feel sick at any time during the study, you should contact the clinic right away. We may ask you to come to the clinic for an examination before your next planned visit. It is very important that you follow the instructions we give you.

Each vaccination visit will last about 4 to 6 hours. Other visits will last about 1 to 2 hours.

At each visit, we will check you for any health changes or problems. We will check you for possible side effects from the vaccine. We will ask how you are feeling and if you have taken any medications. At scheduled study visits, we will draw about 2 to 16 tubes of blood from you, depending on the visit. If any of your test results show a health problem, we will tell you about it as soon as possible. You might need to have extra clinic visits and laboratory tests if you have health changes that we need to check.

If you get sick with an upper respiratory infection (URI), we may take samples from your nose to test for possible causes of the illness, like the flu or RSV. We will use a small disposable sponge that looks like a "Q-tip" to collect these samples. Each sponge is new and sterile, and is safe for use in sensitive parts of the body. You may need to see your primary care provider if you need treatment or follow-up care for your URI.

We will also collect samples from your mouth and nose for research purposes at 2 scheduled study visits. We will use the same or a similar small disposable sponge to collect these samples. These samples are not used to check your health and do not replace routine health care.

Experimental vaccine studies follow a set schedule. The study schedule for your visits allows some flexibility, but it is important that you work with the staff to follow the schedule. You should try to not miss any visits.

MONITORING OF THE STUDY

This study will be monitored by a group of physicians and scientists associated with the NIH. This group will review the study information and will pay close attention to any reactions. If there are serious side effects, injections may be delayed or canceled.

GENETIC TESTING

Some of the blood drawn from you as part of this study will be used for genetic tests. Some genetic tests are done in research studies to see if genetic differences in people cause different types of immune responses. Your blood sample used in these genetic tests will not have your name on it and the results will not be in your medical record. These tests are not used to check your health and we will not tell you the results.

A special genetic test, called HLA typing, may be done by the NIH Clinical Center medical laboratory. These results will be in your medical record but they will not be used to check your health. Any genetic testing, including HLA typing, is for research purposes only. Any genetic information collected or learned about you will be kept confidential. Medical records, including HLA test results, are kept securely. We will not give any genetic information that is in your medical record to anyone without your permission. If HLA typing is done in a research laboratory, the result will not be in your medical record.

STORED SAMPLES

We will collect blood and other bodily fluids including nasal and oral secretions from you during this study. We will keep these samples to study your immune response to the vaccine and for future research to learn more about RSV, vaccines, the immune system, or other medical conditions. Results from the research done from your stored samples are not for medical care and will not be in your medical record.

Labeling of Stored Samples: Your stored samples will be labeled by a special code or number that only the study team can link to you. Any information that could identify you, like your name or date of birth, will be kept as confidential as allowable by law. Even with these protections, there is a small chance that information identifying you will be accidentally given to someone who should not get it.

Future Studies: In the future, other researchers at NIH or outside of NIH may wish to study your stored samples. When your samples are shared, they will be marked with a code. Your samples will not have any information on them that could identify you. Some other information about you, such as your gender, age, health history, or ethnicity may be shared. Any future research done with your samples will be done in a way that protects your rights and privacy.

Your stored samples will be used only for research and will not be sold. The research done with your samples may be used to develop new products in the future but you will not get payment for these products.

HUMAN DATA SHARING

To advance science, it is helpful for researchers to share information they get from studying humans by putting it into shared scientific databases. Researchers can then study the information combined from many studies to learn even more about health and diseases.

If you agree to take part in this study, some of your data will be placed into one or more scientific databases. We will remove identifying information like your name, address, and birth date. The data may then be used for future research and shared broadly for research purposes. Only researchers who are approved to access the database may be able to see and use your information. You will not get any direct benefits from future research that uses your data and information.

You may stop participating in this study at any time and withdraw permission for your individual data, specimens, and health information to be used for additional or future research. You may ask to have your research data destroyed. However, it may not be possible to withdraw or delete data once they have been shared with other researchers.

POSSIBLE STUDY RISKS

***Possible* risks of injections:** Temporary stinging, pain, redness, soreness, itchiness, swelling, or bruising. There is a very small chance of infection.

***Possible* risks of blood drawing:** Pain, bleeding, bruising, feeling lightheaded, or fainting, and rarely, infection at the site where the blood is taken.

***Possible* risks of mucosa sample collection:** Samples collected by rubbing swabs over mucosal surfaces in the mouth or in the nose can cause brief discomfort, or a little bleeding.

***Possible* risks of any vaccine:** Fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired or unwell. Some people have allergic reactions to vaccines. These types of reactions are usually greatest within the first 24

hours after an injection and typically last 1 to 3 days. Over-the-counter medicine, such as acetaminophen, may be used to help relieve these symptoms.

Very rarely, a serious allergic reaction with symptoms like hives, trouble breathing, or sudden weakness may happen shortly after any vaccination. This is called "anaphylaxis" and may be life-threatening. While you are waiting in the clinic after the injections, we will monitor you for anaphylaxis. Treatment for anaphylaxis will be given right away if it happens.

***Possible* risks of the RSV vaccine:** This is the first study to give the RSV vaccine to humans. There may be side effects, even serious or life threatening ones, which we do not know about yet. As of September 09, 2018, 90 subjects have received the RSV vaccine and no safety concerns have been found. We will tell you if we learn about any important new findings or serious side effects during the study that may change your mind about your desire to continue in the study.

***Possible* risks of alum:** Alum is the most common adjuvant used in human vaccines licensed by the FDA. In healthy subjects, side effects are generally mild local reactions at the injection site that may include tenderness, redness, and/or swelling. Another more severe local reaction at the injection site may include lumps under the skin.

***Possible* risks during Pregnancy:** We do not know if getting the study products will affect a fetus. Therefore, women who can get pregnant must agree to use an effective method of birth control starting at least 21 days before getting the first injection until 4 weeks after the last injection. We will discuss effective birth control methods with you. If you are pregnant or want to become pregnant in the next 16 weeks, you cannot participate.

During the study, if you think you might be pregnant, you must tell the clinic staff right away. If you are pregnant, you will not get any more doses of study product. You will be asked to continue with some follow-up visits so that we can check on your health. We will ask you about the outcome of the pregnancy.

***Possible* risks of genetic testing:** Unplanned release of information that could be used by insurers or employers to discriminate against you or your family; discovering a gene that suggests risk of disease for you or your family; discovering unknown family relationships.

***Possible* risks of data sharing:** Information in the shared databases could be linked back to you and used to discriminate against you or your family. State and federal laws provide some protections against genetic and pre-existing conditions discrimination.

***Unknown* risks:** We do not know if the study product will affect how you respond to any RSV infection or RSV vaccine that you may get in the future.

You may not donate blood while taking part in this study and you may not donate blood for one year after the date of your last study injection.

POSSIBLE BENEFITS

This study is not designed to benefit you or to protect you from RSV infection. You and others may benefit in the future from the information that we learn from the study.

COSTS OF PARTICIPATION

There are no costs to you for taking part in this study. You or your health insurance will have to pay for all medical costs for medical care that you get outside of this study. It is possible that you may have some costs that are not covered by the study compensation we give you.

COMPENSATION TO YOU FOR TAKING PART IN THE STUDY

You will get compensation consistent with the NIH policy to help with transportation costs and other expenses that may occur because of study participation. You may have some expenses that are not covered by the compensation provided.

The compensation is: $175 for scheduled visits that include blood drawing only, $275 for vaccination visits, an additional $50 for any visit that includes mucosal sample collection, $25 for finishing all 7 days of an electronic diary, and $75 for clinic visits that do not include blood drawing or mucosal swabs. Total compensation for completion of the study and all injections is estimated to be $2600. Actual compensation is based on the number and type of visits you complete.

You will get your compensation about 2 weeks after each completed visit by direct deposit into a bank account that you specify to the volunteer payment office. Compensation may need to be reported to the Internal Revenue Service (IRS) as taxable income.

REASONS FOR STOPPING YOUR STUDY INJECTIONS

You may not get all of your planned study vaccinations. Reasons for this may include:

- you decide not to get the second injection if this option is offered to you.
- you do not keep appointments or follow procedures.
- you get a serious illness that needs ongoing medical care.
- you have a serious side effect thought to be due to the study vaccine.
- you become pregnant.
- you need to get treatment with a medication that affects your immune system (such as a steroid like prednisone).
- the study is stopped by regulatory agencies, the study sponsor or study investigators. If this happens, we will tell you why.

REASONS FOR REMOVING YOU FROM THE STUDY

You may be taken out of the study without your consent. Reasons for this may include:

- continuing in the study could hurt you,
- you don't follow instructions or keep your appointments, or
- the study is stopped by the NIH, the FDA or other regulatory authorities.

If you agree to take part in this study, it is important for you to keep all of your appointments. Your participation in this study is completely voluntary. You can choose to stop taking part in the study at any time. There is no penalty or loss of benefits for choosing to leave the study.

If you get the first injection but not the second, we will still want you to continue with all planned follow-up visits until the end of the study. It is important that we continue to check your health even if you do not get the second dose.

ALTERNATIVES

This study is not designed to treat or prevent any disease. You may choose not to take part in this study.

CONFLICT OF INTEREST

The NIH research staff is checked yearly for conflicts of interest. You may ask the research team for more information. This study may have investigators who are not NIH employees. Non-NIH investigators are expected to follow the principles of the Protocol Review Guide.

The NIH, including some members of the VRC scientific staff, developed the investigational vaccine being used in this research study. The results of this study could play a role in whether the FDA will approve the vaccine for sale at some time in the future. If approved, the future sale of the vaccine could lead to payments to NIH and some NIH scientists. By U.S. law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development or sale of the product.

CLINICALTRIALS.GOV

A description of this clinical trial will be available on www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the study results. You can search this website at any time.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.
The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the FDA, members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The NIH Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the NIH, the NIH Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the NIH policies. In general, patients are not paid for taking part in research studies at the NIH. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, [redacted] or the Study Coordinator, [redacted]

You may also call the NIH Clinical Center Patient Representative at [redacted]

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

Adult Study Participant's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

	Time
Signature of Adult Participant	Date
Print Name	

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM SEPTEMBER 9, 2019 THROUGH SEPTEMBER 8, 2020.

Signature of Investigator/ Person Obtaining Consent	Date	Signature of Witness	Date
Print Name		Print Name	