Protocol Title: VRC 323: A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of an Influenza H10 Stabilized Stem Ferritin Vaccine, VRCFLUNPF0103-00-VP, in Healthy Adults

NCT: 04579250

Protocol (v2.0 19NOV2020) - Statistical Analysis Considerations located in Section 6 of the Protocol

Version 2.0
November 19 , 2020

VACCINE RESEARCH CENTER

Protocol VRC 323

(NIH 20-I-0145)

TITLE: A PHASE I OPEN-LABEL CLINICAL TRIAL TO EVALUATE THE DOSE, SAFETY, TOLERABILITY AND IMMUNOGENICITY OF AN INFLUENZA H10 STABILIZED STEM FERRITIN VACCINE, VRC-FLUNPF0103-00-VP, IN HEALTHY ADULTS

ABBREVIATED TITLE: H10ssF-6473 FLU VACCINE

IND Sponsor
Vaccine Research Center (VRC)
National Institute of Allergy and Infectious Diseases (NIAID)
Bethesda, Maryland, US

IND 21162

Investigational Product H10ssF-6473 Manufacturer:
Manufactured for VRC by Leidos Biomedical Research, Inc., Frederick, Maryland, US

NIH Principal Investigator: Joseph Casazza, MD, PhD
VRC/NIAID
Building 40, [redacted]
40 Convent Drive
Bethesda, MD 20892
[redacted]

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

Abbreviation	Term
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AoU	assessment of understanding
BMI	body mass index
β-HCG	beta-human chorionic gonadotropin
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
COVID-19	coronavirus disease 2019
cGMP	current Good Manufacturing Practices
CMP	Clinical Monitoring Plan
CRO	contract research organization
DNA	deoxyribonucleic acid
DP	drug product
DTM	Department of Transfusion Medicine
EC	Ethics Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practices
HA	influenza hemagglutinin protein
HIV	human immunodeficiency virus
ICF	Informed Consent Form
ICH	International Council on Harmonisation
ILI	influenza-like illness
IM	Intramuscular
IND	investigational new drug application
IRB	Institutional Review Board
IUD	intrauterine device
LIMS	Laboratory Information Management System
MedDRA	Medical Dictionary for Regulatory Activities
MPA	Medroxyprogesterone acetate
MSD	meso-scale discovery
NA	neuraminidase
NAb	Neutralizing antibody
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH CC	NIH Clinical Center
NOAEL	No Observed Adverse Effect Level
NSAID	nonsteroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator

Abbreviation	Term
PSRT	Protocol Safety Review Team
RBS	receptor binding site
RNA	ribonucleic acid
SAE	serious adverse event
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SUSAR	serious and unexpected suspected adverse reaction
TB	Tuberculosis
ULN	upper limit of normal
UP	Unanticipated Problem
VCMP	Vaccine Clinical Materials Program
VIP	Vaccine Immunology Program
VRC	Vaccine Research Center
WBC	white blood cell

PRINCIPAL INVESTIGATOR PROTOCOL SIGNATURE PAGE

VRC 323: A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of an Influenza H10 Stabilized Stem Ferritin Vaccine, VRC-FLUNPF0103-00-VP, in Healthy Adults

I, the Principal Investigator for the study site indicated above, agree to conduct the study in full accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct the study in compliance with United States (US) Health and Human Services (HHS) regulations (45CFR 46); applicable US Food and Drug Administration (FDA) regulations; standards of the International Council on Harmonization Guidelines for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee (IRB/EC) determinations; all applicable in- country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health) and institutional policies. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. The protocol signature page will be signed for subsequent protocol approvals.

I agree to maintain all study documentation pertaining to the conduct of this study, including but not limited to, case report forms, source documents, laboratory test results, and medication inventory records, per FDA regulation (21 CFR 312.62) and all applicable requirements. No study records will be destroyed without prior authorization from VRC/NIAID.

Publication of the results of this study will be governed by the VRC/NIAID policies. Any presentation, abstract, or manuscript will be made available by the investigators to VRC Leadership for review prior to submission.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____	_____
Name/Title of Principal Investigator	Study Site Name
_____	_____
Signature of Principal Investigator	Date

PRÉCIS

Title: **VRC 323**: A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of an Influenza H10 Stabilized Stem Ferritin Vaccine, VRC-FLUNPF0103-00-VP, in Healthy Adults

Design: This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of VRC-FLUNPF0103-00-VP in 2 regimens. The hypotheses are that the vaccine is safe and tolerable and will elicit an immune response. The primary objective is to evaluate the safety and tolerability of the investigational vaccine in healthy adults. Secondary objectives are related to immunogenicity of the investigational vaccine and dosing regimen

Study Products: The investigational vaccine, VRC-FLUNPF0103-00-VP (H10ssF-6473), was developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID) and is composed of *Helicobacter pylori* non-heme ferritin assembled with influenza virus H10 haemagglutinin (HA) insert to form a nanoparticle displaying eight HA stabilized stems trimers from A/Jiangxi/IPB13/2013 (H10N8) influenza. The vaccine is supplied in single-use vials at a concentration of 180 mcg/mL. VRC-PBSPLA043-00-VP consisting of sterile phosphate buffered saline (PBS) will be the diluent for H10ssF-6473. Prepared study product will be administered intramuscularly (IM) in the deltoid muscle via needle and syringe.

Subjects: Healthy adults between the ages of 18-70 will be enrolled; adults born between 1965 and 1970 will be excluded from the trial.

Study Plan: This study will evaluate the safety, tolerability and immunogenicity of 1 or 2 doses of H10ssF-6473 in a dose-escalation design. In Group 1, the first 3 subjects will receive a single low dose (20 mcg) of H10ssF-6473 on Day 0. If assessed as safe and tolerable two weeks after vaccination of the third subject, enrollment will continue for the additional subjects in Group 1 and begin for Group 2A. For Group 1, the protocol requires 1 vaccination visit, 8 follow-up visits, and a telephone contact after vaccination.

Groups 2A and 2B are stratified by age as shown in the vaccination schema. In Group 2A, the first 3 subjects will receive a higher dose (60 mcg) of H10ssF-6473 on Day 0. If assessed as safe and tolerable two weeks after vaccination of the third subject, enrollment will continue in Group 2A, begin for Group 2B, and subjects may receive the second vaccination at week 16. For Groups 2A and 2B, the protocol requires 2 vaccination visits, 10 follow-up visits, and a telephone contact after each vaccination.

For all groups, solicited reactogenicity will be evaluated using a 7-day diary card. Assessment of vaccine safety will include clinical observation and monitoring of hematological and chemical parameters at clinical visits throughout the study.

VRC 323 Vaccination Schema

Group	Age Cohort	Subjects	Day 0	Week 16
1	18-50	5	20 mcg IM	
2A	18-50	10-15	60 mcg IM	60 mcg IM
2B	55-70	10-15	60 mcg IM	60 mcg IM
Total		25-35*	*Enrollment up to 45 is permitted if additional subjects are needed for safety or immunogenicity evaluations.	

Study Duration: Subjects will be evaluated for 40 weeks following the first vaccine administration.

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1. INTRODUCTION AND RATIONALE

1.1 INTRODUCTION

Influenza virus causes seasonal epidemics and pandemics at irregular intervals that result in significant morbidity and mortality. According to the World Health Organization, the annual global attack rate of influenza is estimated to be 5%–10% in adults and 20%–30% in children; worldwide these annual epidemics result in about 3 to 5 million cases of severe illness and about 250,000 to 500,000 deaths [1]. Domestically, there are an estimated between 9.3 million – 49.0 million cases of influenza illnesses, between 140,000 – 960,000 hospitalizations and between 12,000 – 79,000 deaths annually since 2010 in the United States (US) [2]. The US Centers for Disease Control and Prevention (CDC) estimates that a pandemic influenza outbreak costs the US between $71 billion and $167 billion without counting the financial impact on commerce and society [3].

Influenza is an enveloped, negative single-stranded ribonucleic acid (RNA) virus that belongs to the family *Orthomyxoviridae*. Of the five genera of influenza circulating in nature, only influenza A and B are known to cause epidemics in humans [4].

Influenza A viruses consist of 8 RNA gene segments and are classified based on the antigenicity of their surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA) [5]. The HA subtypes are classified into two groups based on their antigenic properties and their major structural features. Group 1 encompasses the H1a, H1b and H9 clades and Group 2 includes the H3, H7 and H10 clades [6]. There are 18 different HA subtypes (H1 through H18) and 11 NA subtypes known to exist, but only three HA subtypes (H1, H2, from Group 1 and H3 from Group 2) and two NA subtypes (N1 and N2) have caused significant human epidemics [5].

The HA is the predominant viral antigen target for antibody neutralization [7]. HA glycoprotein consists of a globular head domain (which is highly variant in structure among HA subtypes) and a stem domain (which is highly conserved across HA subtypes) [8]. Most of the antibodies produced by the immune system after infection recognize the head domain, whereas the stem domain is recognized by a small population of antibodies [9-11]. The anti-head antibodies are very potent but are strain-specific while the anti-stem antibodies are less potent and are cross-reactive across HA strains [12].

Influenza exhibits genetic flexibility and antigenic variability because of its ability to go through antigenic “drift” (the gradual accumulation of mutations over time) and antigenic “shift” (the replacement of the hemagglutinin gene by reassortment during contemporaneous infection of a host by more than one influenza strain). The emergence of new influenza strains through continuous mutation and reassortment of circulating virus diminishes the effectiveness of annual influenza vaccines [13, 14].

Furthermore, in the US, the current manufacturing process in egg-based systems can lead to lower yields and significant lag times due to virus strain identification and vaccine production, availability, and distribution [15, 16].

These limitations have raised the need for developing a universal influenza vaccine that can provide durable, cross-strain protection against different influenza viruses, with a rapid manufacturing process in which large vaccine quantities could be produced under well-controlled conditions. A universal influenza vaccine would eliminate the need for annual reformulation and revaccination and improve pandemic preparedness [17].

With the goal of developing a universal influenza vaccine, the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) has engineered and optimized stabilized Group 1 and Group 2 influenza HA stem ferritin vaccines that enhance the presentation of the HA stem to the immune system, potentially improving breadth against Group 1 and Group 2 influenza strains [18, 19]. These stabilized Group 1 and Group 2 influenza HA stem ferritin vaccines may inform the development of a universal influenza vaccine and play an important role in the planning and preparation for future influenza pandemics [18, 19].

VRC-FLUNPF0103-00-VP (H10ssF-6473) is an investigational vaccine that has not been administered to humans before this study. The H10ssF-6473 vaccine is composed of *H. pylori* non-heme ferritin assembled with a Group 2 influenza virus H10 HA insert to form a nanoparticle displaying eight HA stabilized stem trimers from an A/Jiangxi/IPB13/2013 (H10N8) influenza virus.

1.2 RATIONALE FOR DEVELOPMENT OF VRC-FLUNPF0103-00-VP, H10SSF-6473

New vaccine platforms and production technologies directed toward the goal of a universal influenza vaccine include cell-culture-based manufacturing processes, novel live attenuated vaccines, recombinant proteins, recombinant DNA-based vaccines, and nanoparticles [15, 16].

Ferritin is a highly conserved, ubiquitous iron storage protein that is found in various species from Archaea, Eukarya, and Bacteria domains [20]. Ferritin particles can be used in vaccines for antigen presentation of influenza HA [21]. Ferritin self-assembles into a nearly-spherical nanoparticle, composed of 24 subunits organized in an octahedral symmetry with a hollow interior, that mimics the structure of the viral antigen and mediates the interaction with the immune system [21, 22]. The advantages of using ferritin as a vaccine platform to improve antigen presentation and immune stimulation against different strains of influenza viruses rely on the ability to obtain higher levels of protein quaternary structures and the capacity to display heterologous antigens on their surface [21, 23]. Furthermore, as the self-assembly process requires no energy and can be manufactured from simple expression vectors (without relying on egg-based systems), vaccine manufacturing timelines could potentially be shortened, improving the response to an influenza pandemic [21].

Investigators at the VRC, NIAID, NIH, Bethesda, MD, US, have identified a non-heme ferritin from *H. pylori* as a protein able to display eight trimeric influenza HA spikes that mimic the physiological HA structure.

Kanekiyo, *et al.* genetically fused the ectodomain of A/New Caledonia/20/1999 HA to *H. pylori* ferritin, creating a vaccine that antigenically resembles the native head and stem domains of the HA viral spikes on the surface of the ferritin spherical core [21]. In preclinical immunogenicity studies, this HA ferritin vaccine elicited two types of broadly neutralizing antibodies: against the highly conserved HA stem and against the conserved receptor binding site (RBS) on the head of the viral HA. The HA stem and the RBS are structures of major interest for the development of a universal vaccine against influenza [24].

Yassine, *et al.* genetically fused the ectodomain of A/New Caledonia/20/1999 HA that lacks the immunodominant head domain to *H. pylori* ferritin, creating a ferritin nanoparticle that antigenically resembles the native stem domain of the HA protein on the surface of the ferritin spherical core [18]. In preclinical immunogenicity studies, this H1 HA stem ferritin vaccine

conferred heterosubtypic protection against H5N1 influenza virus challenge in multiple animal models, indicating that vaccine-elicited HA stem–specific antibodies can protect against diverse Group 1 influenza strains [18].

Both of these HA ferritin vaccines possess the desired structural properties and have demonstrated the capacity to enhance the breadth of neutralizing antibodies in pre-clinical studies when compared to the current commercial trivalent inactivated vaccine containing the same 1999 H1N1 HA [18, 21].

Corbett, *et al*. developed headless Group 2 HA stem nanoparticle immunogens from both H3 and H7 influenza virus subtype and showed that the highly conserved stem region of HA of Group 2 influenza A virus subtypes is a promising target for vaccine elicitation of broad cross-group protection against divergent strains [19].

Based on data reported with the HA stem ferritin vaccine in animal studies (2.1.3) as well as preliminary safety data from 2 prior phase I trial utilizing the *H. pylori* non-heme ferritin genetically fused to an influenza virus H2 HA (1.3.2) or H1 HA stem (1.3.3), it is expected that the H10ssF-6473 vaccine will be safe and immunogenic in this Phase I study in humans.

1.3 BACKGROUND

1.3.1 Human Experience with VRC-FLUNPF0103-00-VP (H10ssF-6473)

This is the first clinical study to test the VRC-FLUNPF0103-00-VP vaccine, and there is no previous human experience with this product. Human experience with VRC-FLUNPF081-00-VP (HA-F A/Sing) and VRC-FLUNPF099-00-VP (H1ssF_3928), which both use this ferritin-based vaccine platform, is provided below.

1.3.2 Previous Human Experience with VRC-FLUNPF081-00-VP (HA-F A/Sing)

The first Phase I clinical trial (VRC 316; NCT03186781) using ferritin particles, the HA-F A/Sing ferritin vaccine, opened to accrual in October 2017. The HA-F A/Sing vaccine is composed of *H. pylori* non-heme ferritin genetically fused to the influenza virus H2 HA to form a nanoparticle that antigenically resembles the native head and stem domains of the HA from A/Singapore/1/57 (H2N2) influenza. Evaluation of HA-F A/Sing included 2 dose groups. The low dose group received a single 20-mcg dose at Day 0. The high dose group received a 60 mcg dose at Day 0 and Week 16.

As of February 13, 2020, the study was fully enrolled and closed to accrual. However, at the time of this summary, data quality assurance and monitoring are in progress, and therefore all reported data are preliminary and should not be considered final.

All 47 subjects, healthy adults 18-70 years of age with the exclusion of those born between 1966 and 1969, have received at least one HA-F A/Sing product administration. The first study enrollment and product administration occurred on October 25, 2017 and the final enrollment occurred on November 26, 2018. All injections with the HA-F A/Sing vaccine have been well tolerated.

Maximum solicited local reactogenicity in the 7 days after HA-F A/Sing administration was reported as mild local pain/tenderness for 7 of 47 (14.9%) subjects. No other local symptoms were reported.

Regarding solicited systemic reactogenicity in the 7 days after HA-F A/Sing product administration, 18 of 47 (38.3%) subjects had one or more systemic signs or symptoms; the most frequently reported AE was mild headache (13/47, 27.7%). Subjects also reported mild malaise (10/47, 21.3%), mild myalgia (8/47, 17.0%), moderate myalgia (1/47, 2.1%), mild chills (2/47, 4.3%), mild nausea (1/47, 2.1%) and mild joint pain (2/47, 4.3%). One subject reported severe fever that started on Day 4 after the first product administration, resolved within 1 day, and coincided with an influenza-like illness.

The most frequently reported unsolicited adverse events (AEs) were anemia (8/47, 17.0%), with a maximum severity of moderate for five subjects (5/47, 10.6%), and upper respiratory tract infection (5/47, 10.6%), with a maximum severity of moderate for one subject (1/47, 2.1%). All these unsolicited AEs were assessed as unrelated to study products. A severe AE of viral infection was reported by one subject 9 days after the initial study injection. The event was assessed as unrelated to study product and resolved with no residual effects three days after onset. The following 3 mild AEs were assessed as related to the HA-F A/Sing vaccine and all resolved with no residual effects: leukocytosis, increased blood alkaline phosphatase, and leukopenia.

One life-threatening SAE of myocardial infarction was reported by a subject at 117 days after product administration. The event was assessed as unrelated to study product and resolved with sequelae.

1.3.3 Previous Human Experience with VRC-FLUNPF099-00-VP (H1ssF_3928)

The second Phase I clinical trial (VRC 321; NCT03814720) using ferritin particles, the H1ssF_3928 ferritin vaccine, opened to accrual in April 2019 and is ongoing. The H1ssF_3928 vaccine is composed of *H. pylori* non-heme ferritin genetically fused to the influenza virus H1 HA to form a nanoparticle that antigenically resembles the native stem domain of the HA from A/New Caledonia/20/1999 (H1N1) influenza. Evaluation of H1ssF_3928 included 2 dose groups. The low dose group received a single 20-mcg dose at Day 0. The high dose group received a 60-mcg dose of product at Day 0 and Week 16.

As of February 6, 2020, 51 healthy adults 18-70 years of age have received at least one injection of the H1ssF_3928 vaccine. At the time of this summary, data quality assurance and monitoring are in progress, and therefore all reported data are preliminary and should not be considered final. All injections with the H1ssF_3928 vaccine have been well tolerated.

Maximum local reactogenicity in the 7 days after H1ssF_3928 administration was reported as none by the majority of subjects (42/51, 82.4%). Nine (9/51, 17.6%) subjects reported mild pain after product administration and no moderate or severe local symptoms have been reported to date.

Regarding solicited systemic reactogenicity in the 7 days after product administration, none was reported by the majority of subjects (37/51, 72.5%). The most frequently reported AE was mild headache (10/51, 19.6%), followed by mild malaise (6/51, 11.8%) and mild myalgia (4/51, 7.8%).

Overall, twenty-one subjects (21/51, 41.2%) have had one or more unsolicited AE with maximum severity being mild for twelve subjects (12/51, 23.5%) and moderate for nine subjects (9/51, 17.6%). Three AEs were assessed as related to study product including: mild abnormal

dreams, mild lymphopenia, and moderate neutropenia, all of which resolved. No SAEs have been reported.

1.4 RATIONALE FOR STUDY PRODUCT DOSE

The VRC 316 and VRC 321 study results show that two dose levels (20 mcg as a single dose and 60 mcg as a repeat dose with a 16-week interval) of HA-F A/Sing and H1ssF_3928 were safe and well tolerated. Doses were also evaluated by preclinical evaluations of H10ssF-6473 as discussed in Section 2.1.3.

In this study, H10ssF-6473 will similarly be evaluated at 20 mcg as a single dose and 60 mcg as a repeat dose with a 16-week interval. The 16-week interval between first and second doses is based on previous VRC studies of a similar interval suggesting that this interval may be optimal for development of an immune response to the vaccine regimen [unpublished data].

1.5 RATIONALE FOR STUDY POPULATION

To assess safety, tolerability, and immunogenicity of the H10ssF-6473 vaccine, healthy subjects 18-70 years of age will be enrolled. The subjects will be stratified into 2 age cohorts (18-50 and 55-70). Subjects born between the years 1965 and 1970 will be excluded based on the likelihood or predictability of potential impact of an initial H3 (group 2 influenza) vs. H1 or H2 (group 1 influenza) exposure. Subjects born after 1970 (younger age cohort) will have likely been initially exposed to either an H3 or H1 influenza; while subjects born prior to 1965 (older age cohort) will have had an initial exposure to an H2 (group 1) influenza.

The age gap in which subjects are ineligible, adults born between January 1, 1965 and December 31, 1970, excludes subjects with unknown predictability of exposure. Stratifying by likely initial influenza exposure may allow for assessment of immune imprinting on response to vaccination in this small trial.

1.6 ASSESSMENT OF VACCINE IMMUNOGENICITY

In this study, specimens to evaluate immunogenicity will be collected at baseline and at specified time points. The primary immunogenicity time points are two weeks after the vaccination for Group 1 and two weeks after each vaccination for Groups 2A and 2B assessed by detection of HA-stem responses to H10 using the Meso-Scale Discovery (MSD) or similar platform, as previously described [25]. Additional assessment of HA stem-specific antibody may be conducted on stored samples obtained throughout the study.

Exploratory evaluations will include measurements of human ferritin antibody and *H. pylori* ferritin antibody. Additional exploratory evaluations may include the detection of neutralizing antibodies by pseudovirus neutralization and functional serological assays, and exploratory B and T cell assays.

Research sample processing prior to immunogenicity testing will be performed by the VRC Vaccine Immunology Program (VIP) in Gaithersburg, MD. which will also perform some of the immunogenicity assays.

Some immunogenicity assays may be performed by VRC laboratories in Bethesda, MD or by approved contract laboratories or research collaborators.

Results from this study are expected to contribute to the fund of knowledge needed for the development of a universal influenza vaccine candidate as well as show proof-of-concept for elicitation of antibody responses by a group 2 influenza HA stem vaccination.

2. STUDY PRODUCT

The study product VRC-FLUNPF0103-00-VP (H10ssF-6473) and the diluent, VRC-PBSPLA043-00-VP (PBS), are manufactured under current Good Manufacturing Practice (cGMP) at the VRC Pilot Plant by Leidos Biomedical Research, Inc., Frederick, MD.

2.1 VRC-FLUNPF0103-00-VP, H10ssF-6473

The VRC-FLUNPF0103-00-VP (H10ssF-6473) vaccine is composed of the HA stem domain from A/Jiangxi/IPB13/2013 (H10N8) influenza genetically fused to the ferritin protein from *H. pylori*. Purified H10ssF-6473 displays eight well-formed HA trimers that antigenically resemble the H10 stem viral spikes.

2.1.1 Product Composition and Formulations

VRC-FLUNPF0103-00-VP is a sterile aqueous buffered solution aseptically filled into 3 mL single-dose glass vials. Each vial contains 0.7 ± 0.1 mL at a concentration of 180 ± 36 mcg/mL in formulation buffer composed of 20 mM sodium phosphate, pH 7.2, 100 mM sodium chloride, 5% w/v sucrose, 0.01% w/v Pluronic F-68 (Poloxamer 188). Vials are intended for single use only and do not contain preservative.

2.1.2 In-Use Stability Data

H10ssF-6473 drug product (DP) (Lot No. 19-411) was evaluated under simulated clinical storage and administration conditions for varying time points. DP stability was assessed from unopened, thawed vials and from prepared syringes at concentrations of ~180 mcg/mL and ~60 mcg/mL. Minimal changes in the strength, purity and potency of H10ssF-6473 samples were observed after storage in vials or syringes (irrespective of concentration) for all time points tested. These data support the proposed clinical handling procedure outlined in the protocol. Prepared syringes may be stored cumulatively at 5°C ± 3°C for 24 hours and at temperatures up to 25°C ± 2°C for maximum 4 hours, including administration time.

H10ssF-6473 DP (Lot No. 19-411) has been placed on a stability protocol at the long-term storage condition of -35°C to -15°C at the VRC Pilot Plant. At intervals as specified in ICH Guideline Q5C, DP will be assessed for potency, purity, strength, sterility, and identity.

2.1.3 Preclinical Studies with H10ssF-6473

To evaluate safety of H10ssF-6473, a GLP toxicology study was conducted at IITRI, Chicago, IL. New Zealand White rabbits were vaccinated intramuscularly (IM) once every three weeks with developmental, GMP process-representative material for three injections (Table 1). Toxicity and reversibility of effects were assessed after acute and recovery time points for three dose groups of 10 rabbits per sex (M/F) per group.

Table 1: H10ssF-6473 Toxicology Study Schema

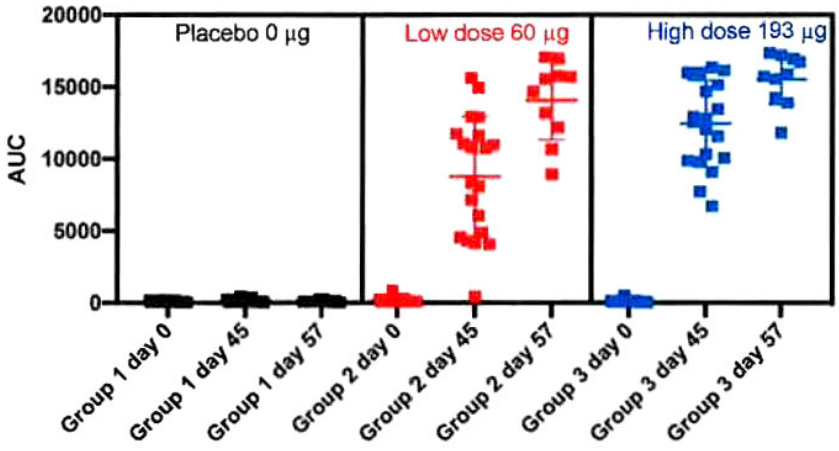
Group #	Treatment Description	H10ssF-6473 Dose Level	Group Population	Necropsied Day 45	Necropsied Day 57
1	Control (diluent)	0	10 M/10 F	5 M/5 F	5 M/5 F
2	Low Dose Vaccine	60 mcg	10 M/10 F	5 M/5 F	5 M/5 F
3	High Dose Vaccine	193 mcg	10 M/10 F	5 M/5F	5 M/5 F

All rabbits received injections on Study Days 1, 22, and 43. Five rabbits per sex per group were necropsied on Study Day 45 (terminal necropsy), two days after the last dose, and five rabbits per sex per group were necropsied on Study Day 57 (recovery necropsy), after a 14-day recovery period. Experimental endpoints included moribundity/mortality, physical examinations and clinical signs of toxicity, injection site reactogenicity (Draize scoring), body weights, food consumption, body temperature measurements, ophthalmology, clinical pathology parameters (clinical chemistry, hematology, and coagulation), immunoassay analysis, organ weights, gross pathology at necropsy, and microscopic pathology.

The vaccine induced a dose-dependent antibody response in vaccinated rabbits (Figure 1). Area Under the Curve (AUC) was individually calculated for each rabbit at each timepoint using GraphPad Prism by plotting the Electrochemiluminescence (ECL) on the y axis and serum dilutions on x axis.

All animals in the low and high dose groups had substantially higher antibody levels on both Day 45 and Day 57 compared to the controls. Antibody levels were both dose- and time-dependent, as higher levels were observed in high-dose animals compared to the low dose animals at both time points, and the highest levels were observed in high-dose animals on Day 57, compared to Day 45.

Figure 1: Summary of H10ssF-6473 Immunogenicity Data for All Animals



Treatment with H10ssF-6473 was very well tolerated as there were no deaths, treatment-related adverse clinical signs, or evidence of injection site reactogenicity. No treatment-related or

toxicologically significant differences were noted for body weights, body weight changes, food consumption, body temperatures, ophthalmology, clinical pathology parameters (clinical chemistry, hematology, and coagulation), or organ weights.

There were no test article-related gross or microscopic findings noted during the study. Inflammatory cell infiltrates in the muscle of the injection site were present at low incidence in the left (most recent) injection site. As this finding followed administration of an immunogenic substance, it was anticipated.

In summary, IM treatment with the H10ssF-6473 vaccine at doses of 60 and 193 mcg for three injections on Study Days 1, 22, and 43 resulted in no treatment-related, toxicologically significant, or adverse findings. The No Observed Adverse Effect Level (NOAEL) was therefore determined to be the highest dose administered, 193mcg.

To support use of H10ssF-6473 in a clinical trial, proof of concept studies were conducted in animal models to measure immunogenicity and protective efficacy against lethal influenza challenge. Together, these data showed that H10ssF-6473 was immunogenic in mice and NHPs and offered protection against challenge.

Initial proof of concept studies were performed with research grade material administered with Sigma Adjuvant System (SAS) adjuvant. Mice were immunized with a two mcg dose of HssF-6473 at Weeks 0, 4, and 8. Eight weeks after the last vaccination, they were challenged with influenza virus at 25x LD50. Mice were protected at 9/10, 8/10, or 10/10 from challenge with an H3N2, an H7N9, or an H10N8 (homologous) virus, respectively. In contrast, all control mice succumbed to infection by Day 8 post challenge. These data showed that H10ssF-6473 was immunogenic in mice, protected completely from homologous H10 influenza challenge, and protected substantially from heterosubtypic Group 2 H3 and H7 influenza challenge.

The immunogenicity of development grade H10ssF-6473, manufactured by a GMP-representative process, was compared in a mouse model to research grade material with doses of 2 or 50 mcg of H10ssF-6473, administered with or without adjuvant SAS. Administered in three doses at Weeks 0, 4, and 8, development grade H10ssF-6473 without adjuvant elicited sera antibodies that bound to H10 HA, and titers rose with number of immunizations. In addition, sera from H10ssF-6473-immunized mice neutralized parental virus in a pseudovirus neutralization assay.

Evaluation of H10ssF-6473 in non-human primates has also been conducted to assess immune responses in a model that potentially more closely approximates human responses to influenza antigens. NHPs were immunized with 50 mcg research grade H10ssF-6473 plus Addavax™ adjuvant at Weeks 0, 4, and 10 with sera drawn two weeks after each immunization. After the third immunization, animals showed specific antibody responses by ELISA against H10 > H7 > H3 hemagglutinin. Sera from the H10ssF-6473-immunized animals also neutralized homologous H10N8 or heterosubtypic H7N9 virus in a reporter virus-based microneutralization assay. In contrast, pre-immune sera from these NHPs showed no binding to group 2 HAs nor neutralization capacity in the pseudovirus assay.

3. STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVES

- To evaluate the safety and tolerability of the VRC-FLUNPF0103-00-VP vaccine, administered as a single dose at 20 mcg IM via needle and syringe on Day 0 to healthy adults.
- To evaluate the safety and tolerability of the VRC-FLUNPF0103-00-VP vaccine, administered at 60 mcg IM via needle and syringe to healthy adults by repeat dosing on Day 0 and Week 16 for a total of 2 injections.

3.2 SECONDARY OBJECTIVES

- To evaluate antibody responses to the VRC-FLUNPF0103-00-VP vaccine administered as a single dose at 20 mcg IM via needle and syringe at two weeks after injection.
- To evaluate antibody responses to the VRC-FLUNPF0103-00-VP vaccine administered as repeat dosing at 60 mcg IM via needle and syringe at two weeks after each injection.

3.3 EXPLORATORY OBJECTIVES

- To evaluate the specificity and functionality of vaccine-induced antibodies and the immune response at various timepoints throughout the study.
- To evaluate the frequency, magnitude, and specificity of B-cell and/or T-cell responses at various time points throughout the study.

4. SUBJECT POPULATION AND CLINICAL PROCEDURES

This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of the VRC-FLUNPF0103-00-VP vaccine in two dose regimens in healthy adults. The study schema is shown in **Table 2**. The hypotheses are that the vaccine is safe, tolerable, and will induce an antibody response to the HA stem of influenza virus subtype H10. The study will be conducted by the VRC Clinical Trials Program at a single site (Vaccine Evaluation Clinic (VEC)) in the NIH Clinical Center (NIH CC), Bethesda, MD.

Table 2: Study Schema

VRC 323 Vaccination Schema				
Group	**Age Cohort**	**Subjects**	**Day 0**	**Week 16**
1	18-50	5	20 mcg IM	
2A	18-50	10-15	60 mcg IM	60 mcg IM
2B	55-70	10-15	60 mcg IM	60 mcg IM
Total		25-35*	*Enrollment up to 45 is permitted if additional subjects are needed for safety or immunogenicity evaluations.	

4.1 STUDY POPULATION

All inclusion and exclusion criteria must be evaluated for eligibility.

4.1.1 Inclusion Criteria

***A subject must meet all of the following criteria**:*

1. Healthy adults between the ages of 18-70 years (excluding adults born between January 1, 1965 and December 31,1970)
2. Based on history and examination, in good general health and without history of any of the conditions listed in the exclusion criteria
3. Received at least one licensed influenza vaccine from 2015 to the present
4. Able and willing to complete the informed consent process
5. Available for clinic visits for 40 weeks after enrollment
6. Willing to have blood samples collected, stored indefinitely, and used for research purposes and genetic investigations.
7. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
8. Physical examination and laboratory results without clinically significant findings and a Body Mass Index (BMI) ≤ 40 within the 56 days before enrollment

Laboratory Criteria within 56 days before enrollment

9. White blood cells (WBC) and differential within institutional normal range or accompanied by the site Principal Investigator (PI) or designee approval
10. Total lymphocyte count ≥ 800 cells/uL
11. Platelets = 125,000 – 500,000 cells/uL
12. Hemoglobin within institutional normal range or accompanied by the PI or designee approval
13. Serum iron within institutional normal range or accompanied by the site PI or designee approval
14. Serum ferritin within institutional normal range or accompanied by the site PI or designee approval
15. Alanine aminotransferase (ALT) ≤ 1.25 x institutional upper limit of normal (ULN)
16. Aspartate aminotransferase (AST) ≤ 1.25 x institutional ULN
17. Alkaline phosphatase (ALP) <1.1 x institutional ULN
18. Total bilirubin within institutional normal range, except when otherwise consistent with Gilbert's syndrome
19. Serum creatinine ≤ 1.1 x institutional ULN
20. Negative for HIV infection by an FDA-approved method of detection

Criteria applicable to women of childbearing potential:

21. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) on the day of enrollment
22. Agrees to use an effective means of birth control from at least 21 days prior to enrollment through the end of the study

4.1.2 Exclusion Criteria

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

1. Breast-feeding or planning to become pregnant during the study

Subject has received any of the following substances:

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 allergy treatment with allergen immunotherapy with antigen injections, unless on maintenance schedule
8. Current anti-TB prophylaxis or therapy
9. Previous investigational H10 influenza vaccine
10. Receipt of a licensed influenza vaccine within 6 weeks prior to enrollment

Subject has a history of any of the following clinically significant conditions:

11. Serious reactions to vaccines that preclude receipt of the study vaccination as determined by the investigator
12. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema
13. Asthma that is not well controlled
14. Diabetes mellitus (type I or II), with the exception of gestational diabetes
15. Thyroid disease that is not well controlled
16. Idiopathic urticaria within the past year
17. Autoimmune disease or immunodeficiency
18. Hypertension that is not well controlled (baseline systolic > 140 mmHg or diastolic > 90 mmHg)
19. 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

20. Malignancy that is active or history of malignancy that is likely to recur during the period of the study
21. 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
22. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen
23. Guillain-Barré Syndrome
24. Previous or current infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) documented by polymerase chain reaction (PCR) test
25. Any medical, psychiatric, social condition, occupational reason or other responsibility 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 INCLUSION OF VULNERABLE SUBJECTS

4.2.1 Children

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

4.2.2 NIH Employees

NIH employees and members of their immediate families may participate in this protocol. We 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 participant'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. Protocol study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

4.3 CLINICAL PROCEDURES AND EVALUATIONS

Evaluation of this vaccine will include laboratory tests, medical history, physical assessment by clinicians, and subject self-assessment recorded on a diary card for 7 days after each injection. Potential adverse reactions will be further evaluated prior to continuing the vaccination schedule.

In response to the coronavirus disease 2019 (COVID-19) pandemic and changing information related to testing, all NIH CC epidemiologic and testing guidelines will be followed in the study

conduct.

The schedule of study evaluations is described in this section and shown in table format in APPENDIX I: SCHEDULE OF EVALUATIONS.

4.3.1 Recruitment and Retention Strategies

Study enrollments will be conducted at the NIH Clinical Center. Study subjects will be recruited through on-site and off-site Institutional Review Board (IRB)-approved advertising done through the VRC's screening protocol, VRC 500 (NCT 01375530). Effort will be made to include women and minorities in proportions similar to that of the community from which they are recruited.

4.3.2 Costs

There are no costs to subjects for their participation in this trial.

4.3.3 Compensation

Subjects will be compensated for time and inconvenience in accordance with the standards for compensation of the NIH Clinical Research Volunteer Program. The compensation per visit will be $315 for visits that include injections and $200 for clinic visits that include a blood draw for all Groups. For Groups 2A and 2B the compensation per visit will be $200 for clinic visits that include a blood draw and oral mucosal sample collection for antibody analysis. Any visit that includes nasopharyngeal swabs for diagnostic purposes for all Groups will result in an additional $55 in compensation. The compensation for any clinic visits that does not include a blood draw or mucosal sample collection will be $85. The compensation for timely completion of the electronic diary card will be $25. Compensation will be $285 for apheresis, if performed. The total compensation for the subject is based on the number of study clinic visits, injections completed, and if optional research blood collections are performed and the electronic diary cards are submitted on time.

The approximate total compensation for Group 1 is $1,940. Approximate total compensation for Groups 2A and 2B is $2,735 without apheresis and $2,820 with apheresis. Subjects will receive compensation by direct deposit approximately 1 or 2 weeks after each completed visit. Compensation may need to be reported to the Internal Revenue Service (IRS) as taxable income.

4.3.4 Screening

Screening for this study will be completed through the VRC's screening protocol, VRC 500 (NIH 11-I-0164, NCT01375530) used for all VRC IND studies conducted at the NIH Clinical Center. The evaluations and sample collections included in screening are a medical history, physical exam, laboratory tests needed to confirm eligibility, and pregnancy test for females of reproductive potential.

If screening evaluations suggest a current concerning health condition or infection, then appropriate laboratory tests may be conducted to evaluate these conditions. Additional assessments of health may be conducted at screening based on clinical judgment. Screening evaluations for specific eligibility criteria (4.1) must be completed within the time interval specified prior to enrollment for the given parameter and may be repeated, as needed, to confirm eligibility.

A nasopharyngeal swab to evaluate for current SARS-CoV-2 infection must be collected no more than 4 days prior to 1) enrollment/first product administration and 2) each study additional product administration. The testing result must be negative for a subject to participate in the study.

Research blood samples may be collected anytime during screening through enrollment and will not be subject to the "56-day prior to enrollment" restriction.

Subjects who are not up to date on standard vaccinations may receive these, if available, during their participation in the screening protocol or at a later date during study participation.

The informed consent form (ICF) will be reviewed, and counseling related to pregnancy prevention will be provided. As part of the informed consent process, an Assessment of Understanding (AoU) will be completed in association with enrollment into VRC 323. Records will be kept documenting the reason that screened subjects do not enroll.

4.3.5 Study Schedule

The Schedule of Evaluations in APPENDIX I: SCHEDULE OF EVALUATIONS provides details on the study schedule, the permitted windows for completing the visits, and the evaluations to be performed at each visit. The visit schedule is based on intervals of time after each study injection (APPENDIX I: SCHEDULE OF EVALUATIONS). The clinicians will discuss the target dates and timing of the study vaccination(s) and sample collections with each subject before completing enrollment to help ensure that subjects can comply with the projected schedule.

After enrollment, deviations from the visit windows are discouraged and will be recorded as protocol deviations but are permitted at the discretion of the PI (or designee) in the interest of completing the vaccination schedule and obtaining subject safety and immunogenicity evaluations.

4.3.6 Enrollment and Study Day 0

Day 0 is defined as the day of protocol enrollment and first injection for all groups. 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 evaluations are within the specified window prior to Day 0 as it is described in the Schedule of Evaluations (APPENDIX I: SCHEDULE OF EVALUATIONS). 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.

The study has staged enrollment with required interim safety reviews, as described in Criteria for Dose Escalation and Dose Continuation, for dose escalation, and dose continuation. In Group 1, the first three subjects will be enrolled with no more than one subject per day. Following dose escalation review, Group 1 can continue enrollment and three subjects will be enrolled directly in Group 2A with no more than one subject per day. An interim safety review, two weeks after vaccination of the third subject, will determine whether enrollment can continue in Group 2A and can begin in Group 2B.

Subjects will be stratified by age into Groups 2A and 2B.

The study group assignment in the database will be set up prior to opening the study to accrual. The group assignment is known to the staff and subject before completing the electronic enrollment into the study on Day 0. Any subject who receives at least one study injection will be expected to continue with follow-up through the end of the study.

4.3.7 Acceptable and Effective Methods of Birth Control

Women of reproductive potential are required to agree to have used an effective method of birth control beginning 21 days prior to enrollment and agree to continuing use of effective birth control through end of study.

Acceptable and effective methods of birth control for women of reproductive potential in this study include: abstinence (no sex) with male partners, birth control pills or patch, condoms, Medroxyprogesterone acetate (MPA) injection, diaphragm or cervical cap, intrauterine device (IUD), Implant (Nexplanon®), NuvaRing®, partner has vasectomy, or use of spermicides.

4.3.8 Vaccine Administration

All study injections will be completed according to the assigned group and will be administered IM in the deltoid muscle. Scheduled blood collection must be completed before vaccinations.

A negative test result for SARS-CoV-2 infection by PCR confirmed at no more than 4 days prior to each product administration is required to proceed with product administration.

On the injection day (prior to injection), study subjects will be clinically evaluated and samples will be collected as per Schedule of Evaluations (APPENDIX I: SCHEDULE OF EVALUATIONS). A subject who arrives at the clinic with fever or evidence of an acute illness that precludes administration of the vaccine may be rescheduled within the allowed study visit window.

Pregnancy test results for women of reproductive potential must be obtained on each injection day prior to the study injection and the results must be negative to proceed.

When choosing an arm 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.

4.3.9 Post-Product Administration Follow-Up

All subjects will be observed for a minimum of 30 minutes following each injection. Vital signs (temperature, blood pressure, pulse and respiratory rate) and inspection for injection site reactogenicity will be performed after each injection.

In keeping with the NIH CC 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.

4.3.10 Solicited Adverse Events (Reactogenicity)

Subjects will be given a “Diary Card” (paper and electronic-based available), a thermometer, and a measuring device. Subjects will use the diary card to record temperature, local and systemic symptoms, and concomitant medications daily for 7 days after each injection. Subjects will be provided training on diary completion and proper usage of the thermometer to measure

temperature and the measuring device to measure injection site symptoms. While subjects will be encouraged to use the secure electronic database, they will 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 on paper, the paper diary card will be the source document. When neither paper nor electronic diary is available from the subject, the study 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: generalized symptoms of unusually tired/feeling unwell; muscles aches (other than at injection site); headache; chills,; nausea; joint pain; and injection site symptoms of pain/tenderness, redness, swelling, pruritus, and bruising. Subjects will also record the day's highest measured temperature and measurement of largest diameter for redness, swelling, and bruising at injection site.

Follow-up on subject well-being will be performed by telephone on the first day following each injection and by clinic visits as shown in the Schedule of Evaluations (APPENDIX I: SCHEDULE OF EVALUATIONS). Subject diaries will be reviewed by a clinician for accuracy and completeness at follow-up visits.

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. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

4.3.11 Follow-Up through End of Study

Study follow-up will continue via clinic visits through 40 weeks following the first vaccine administration. Refer to 4.5 which describes the criteria for discontinuing product administration and/or study participation.

4.3.12 Mucosal Sample Collection

Throughout the study, nasopharyngeal swabs for diagnostic purposes will be requested from all subjects who meet criteria for influenza-like illness (ILI) as defined in 5.1.

Oral mucosal samples for research purposes will be collected only for Groups 2A and 2B at specified study visits according to the Schedule of Evaluations in Appendix I. These samples will be used to evaluate the mucosa immune response induced by immunization with the study product. A small ophthalmic sponge designed for clinical use will be used to collect oral mucosal samples. These samples are for research purposes only and are not used for evaluating subject health.

4.3.13 Blood Sample Collection

At intervals throughout the study, blood will be drawn for safety and immunologic assays. Blood will be drawn from the arm veins of subjects by standard phlebotomy procedures. Total blood volume drawn from each subject will not exceed the NIH CC guidelines.

4.3.14 Apheresis

Group 2A and 2B subjects will be offered apheresis as an optional procedure at Visit 09 in order to collect blood cells of special interest for research. The apheresis procedure will be carried out by trained NIH Department of Transfusion Medicine (DTM) medical staff using automated cell separator devices.

Apheresis performed for this protocol will be performed solely for research purposes. All study subjects will be treated according to standard DTM whole blood and apheresis donation policies and procedures. Prior to the scheduling apheresis, the subject must have a venous assessment performed by the DTM staff.

In order to undergo apheresis procedures, a subject must meet the apheresis eligibility criteria as described in 4.3.15 and have no medical contraindications, as determined by the DTM staff. A VRC study clinician will complete a checklist for apheresis eligibility before referring a subject for the procedure.

Prior to beginning the apheresis procedure, a study clinician may request in advance that other laboratory samples be collected as needed to monitor the well-being of the subject or if needed by a research laboratory. In addition, for women of reproductive potential, a pregnancy test by blood or urine will be performed by a VRC study clinician within 72 hours prior to the apheresis procedure. Results must be negative to proceed with apheresis.

The Dowling Apheresis Clinic staff at the NIH CC routinely performs a hemoglobin test prior to initiating apheresis, per DTM Apheresis Clinic standard policies. If a subject is found to have a hemoglobin value less than permitted by the Apheresis Clinic, then the apheresis will not be initiated, and the ordering provider will be notified.

In this study, the procedure will require two antecubital venous access sites and will involve processing 1 to 4 liters of whole blood. The expected mononuclear cell yield is approximately 0.5 to 1.0 x 10^9 cells per liter processed, and the apheresis device can process about 2 to 3 liters per hour. Thus, 1 to 2 hours are required to process 1 to 4 liters of blood and obtain about 1 to 4 x 10^9 leukocytes. The packed red cell loss during the procedure is the equivalent of a 6 mL blood draw; this is the volume that will be used for the purposes of calculating cumulative blood draw when apheresis is performed.

During or following an apheresis visit, if there is any concern about the well-being of the subject, the DTM clinic may conduct appropriate medical evaluations by history-taking, physical examination, laboratory tests, and/or other testing.

Research blood samples will be processed and stored at VIP or a collaborating research laboratory. Stored samples may be used later to further evaluate immune responses and to elucidate genetic factors associated with immune response.

4.3.15 Apheresis Eligibility Criteria

Subject must meet all of the following criteria:

- Afebrile (temperature $\leq 37.5^0$C)
- Weight ≥110 pounds
- Adequate bilateral antecubital venous access

- Hemoglobin ≥ 12.5 g/dL for females; ≥ 13.0 g/dL for men
- Platelets > 150,000 cells/uL
- No cardiovascular instability as indicated by: a) history of medically significant cardiac arrhythmia within the last 12 months, or b) ischemic cardiovascular disease within the last 12 months, or c) heart rate outside of the 50 - 100 beats/minute interval (on 3 successive readings), or d) blood pressure greater than 180 mmHg (systolic) or 100 mmHg (diastolic) on 3 successive readings
- No current lung or kidney disease
- No known coagulation disorder
- No sickle cell disease
- No active or chronic hepatitis
- No intravenous injection drug use in the past 5 years
- Not breast feeding
- Negative β-HCG pregnancy test (urine or serum) performed by a VRC study clinician within 72 hours prior to the apheresis procedure

4.3.16 Concomitant Medications

Routine prescription medications in use at the time of enrollment will be entered in the database. Subsequently, the concomitant medications that will be recorded or updated in the database are those associated with an AE requiring expedited reporting or the development of a new chronic condition requiring ongoing medical management. Anti-viral medications taken during influenza or influenza-like illnesses will be recorded in the database. Receipt of an 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). Inclusion of a concomitant medications in the database may also be determined at the discretion of the PI. Otherwise, concomitant medications taken throughout the study will be recorded in the subject's study chart and general medical record but will not be recorded in the database.

4.4 Criteria for Dose Escalation and Dose Continuation

There will be two interim safety reviews in this study. The Protocol Safety Review Team (PSRT, Section 8.7.1) will conduct an interim safety data review before dose escalation or repeat dosing may occur. The PSRT must assess the data as showing no significant safety concerns before enrollment of the next dose level and repeat dosing at the same level may proceed.

Enrollment will begin in Group 1 (20 mcg of VRC-FLUNPF0103-00-VP) with no more than one subject enrolled per day for the first 3 subjects. Two weeks after vaccination of the third subject, there will be an interim safety review of available data for three subjects to determine whether to continue enrollment in Group 1 and to proceed to the next dose level. If the 20 mcg dose of VRC-FLUNPF0103-00-VP is assessed as safe, enrollment can continue for Group 1 and begin for Group 2A.

In Group 2A (60 mcg of VRC-FLUNPF0103-00-VP), enrollment will continue with no more than one subject enrolled per day for the first three subjects only. Two weeks after vaccination of the third subject in Group 2A, there will be an interim safety review of the available data for three subjects to determine if the 60 mcg dose of VRC-FLUNPF099-00-VP is assessed as safe. If assessed as safe, recommendations may include to continue enrollment in Group 2A and begin enrollment for Group 2B, also subjects in Group 2A and 2B may receive a second vaccination at week 16. Additional subjects may be enrolled into a group in order to have the requisite data for at least 3 subjects, if first vaccinations are not completed or there are discontinuations from the study before there are sufficient data to conduct the dose escalation and/or continuation review for that group.

Consultation with the IRB and FDA, if needed, as per study pause criteria (Section 4.6) 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 reassessment for safety before proceeding to a higher dose level and repeat dosing at the same dose level.

4.5 Criteria for Discontinuing Study Injections or Protocol Participation

All subjects who received at least one product administration will be encouraged to stay on the study and continue a follow-up for safety. Decisions by the PI or designee to discontinue study injections or protocol participation for a subject will be made with the following criteria.

4.5.1 Discontinuation of Study Injections

A subject will be discontinued from receiving study product for the following reasons:

1. Subject voluntarily withdraws;
2. Pregnancy;
3. Grade 3 unsolicited AE assessed as possibly, probably or definitely related to study product;
4. Grade 4 AE assessed as related to study product;
5. An SAE of any grade assessed as related to study product;
6. Immediate hypersensitivity reaction associated with study product;
7. Intercurrent illness that is not expected to resolve prior to the next scheduled study injection;
8. 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 injections may continue per investigator discretion if the next dose occurs at least 2 weeks following completion of glucocorticoid treatment
9. Repeated failure to comply with protocol requirements;
10. The study PI assesses that it is not in the best interest of the subject to continue the vaccination schedule.

Group 2A and 2B subjects who do not receive the second injection as scheduled are expected to continue follow-up according to the Schedule of Evaluations for Group 1 subjects, except that research sample collections will be discontinued for pregnant women or others for whom it is

contraindicated.

4.5.2 Discontinuation from Protocol Participation

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

- Subject voluntarily withdraws
- Subject develops a medical condition that is a contraindication to continuing study participation
- The IND Sponsor or regulatory authority stops the protocol
- The IND Sponsor or PI assesses that is it 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

4.6 CRITERIA FOR PAUSING AND RESUMING THE STUDY

4.6.1 Plan for Pausing the Study

The PI and Protocol Safety Review Team (PSRT) will closely monitor and analyze study data as they become available and will make determinations regarding the presence and severity of AEs. The administration of study injections and new enrollments will be paused and the IND Sponsor will be promptly notified according to the following criteria:

- **One** (or more) subject experiences a **SAE** or **Grade 4 AE** assessed as related to study product.
- **Two** (or more) subjects experience the same **Grade 3 unsolicited AE** assessed as possibly, probably or definitely related to study product.

Self-limited solicited reactogenicity that is not an SAE will not be counted towards pause criteria.

4.6.2 Plan for Review of Pauses and Resuming the Study

The IND Sponsor, with participation by the PI and PSRT, will conduct the review and make the decision to resume, amend or close the study and notify the IRB accordingly. 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. The pause criterion for the SAE will continue to apply.

The administration of study injections and new enrollments would resume only if review of the events that caused the pause resulted in a recommendation to permit further study injections and enrollments. Safety data reports and changes in study status will be submitted to relevant regulatory authorities in accordance with Section 5 and institutional policy.

5. SAFETY AND ADVERSE EVENT REPORTING

5.1 ADVERSE EVENTS

The term "Adverse Event" (AE) is defined as any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research,

whether or not considered related to the subject's participation in the research. In the context of FDA-required reporting, an AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Each AE will be graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Food and Drug Administration Guidance – September 2007 (APPENDIX II: ASSESSMENT OF RELATIONSHIP TO VACCINE AND GRADING SEVERITY OF ADVERSE EVENTS). The following guidelines will be used to determine whether or not an AE is recorded in the study database:

1. Solicited AEs (i.e., reactogenicity parameters as defined in 4.3.10) will be recorded without attribution assessments by the subject on paper or an electronic diary card for 7 days after each injection. If the paper diary card is completed by subject, data will be transcribed by a clinician into the study database. Clinicians will follow and collect resolution information for any reactogenicity symptoms that are not resolved within 7 days.
2. All unsolicited AEs will be recorded with attribution assessments in the study database from receipt of the first study injection through completion of the 4-week visit that follows each study injection. At other periods between injections and following the 4-week post-injection visit after the second vaccination through the last study visit, only SAEs (5.2), new chronic medical conditions, and influenza or influenza-like illness (ILI) will be recorded.
3. Cases of influenza or influenza-like illness (ILI) will be evaluated as follows:

   ILI is defined as fever (temperature of 100^0F [37.8^0C] or greater) and a cough and/or sore throat in the absence of a known cause other than influenza. Collection of nasopharyngeal swabs will be used for laboratory confirmation of influenza by PCR in subjects who meet criteria for ILI.

   Subsequently, results of any reported laboratory testing for identification of pathogens will be included for cases meeting initial criteria for ILI. The severity of illness in subjects with laboratory confirmed influenza illness will be recorded on a case report form rather than on an AE form.

5.2 SERIOUS ADVERSE EVENTS

The term "Serious Adverse Event" (SAE) is defined in 21 CFR 312.32 as follows: "An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or the 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 or suspected adverse reaction that represents an immediate risk of death to the subject. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. Similarly, a hospital admission for an elective procedure is not considered a SAE.

5.3 ADVERSE EVENT REPORTING TO THE IND SPONSOR

AEs that meet SAE criteria must be reported and submitted by the clinical site on an expedited basis to the IND Sponsor, VRC/NIAID/NIH, according to sponsor guidelines as follows:

- Results in death;
- Is life threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect in the offspring of a study subject; OR
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the 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).

In addition, any event, regardless of severity, which in the judgment of an investigator 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 data entry into the database, which triggers an alert to the IND Sponsor Medical Officer. Within 3 working days, a written summary by the investigator should be submitted to the IND Sponsor.

In order for the IND Sponsor to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 and/or 15 calendar days, the investigator must submit additional information as soon as it is available.

5.4 IND SPONSOR REPORTING TO THE FDA

The IND Sponsor is responsible for making the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs) as defined in 21 CFR 312.32. The following definitions apply:

- *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 (refer to Risks in 8.5.1) 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 per 21 CFR 312.32 as soon as possible but not exceeding 7 calendar days for unexpected fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events. IND Safety Reports will also 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.

5.5 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
- New information that might affect the willingness of subjects to enroll or continue participation in the study

5.5.1 Unanticipated Problem

An Unanticipated Problem (UP) is defined as any incident, experience, or outcome that meets all 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.

5.5.2 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, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects 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 or 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.

5.5.3 Protocol Deviation

A Protocol Deviation (PD) is defined as any change, divergence, or departure from the IRB-approved research protocol and is further characterized as major or 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.

5.5.4 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.

5.5.5 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.

5.5.6 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.

5.5.7 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.

5.5.8 Annual Reporting to the 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

6.1 OVERVIEW

This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of VRC-FLUNPF0103-00-VP in two dose regimens. Study objectives can be found in STUDY OBJECTIVES.

6.2 SAMPLE SIZE AND ACCRUAL

Recruitment will target between 25 to 35 healthy adult participants 18 to 70 years of age. Adults born in 1965 through 1970 will be excluded from the trial. Up to 45 participants may be enrolled if deemed necessary for safety or immunogenicity evaluations. Group 1 will target 5 healthy adults, 18 to 50 years of age born after 1970. Groups 2A and 2B will target 10-15 healthy adult participants each, with study groups corresponding to 18 to 50 years of age born after 1970 and 55 to 70 years of age born between 1950-1965, respectively.

6.3 ENDPOINTS

6.3.1 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 injection and safety evaluated by clinical visits for 40 weeks following the last vaccine administration. SeeClinical Procedures and Evaluations and APPENDIX I: SCHEDULE OF EVALUATIONS for details and specified time points. The following parameters will be assessed for all study groups:

- Occurrence of solicited local reactogenicity symptoms for 7 days after each injection
- Occurrence of solicited systemic reactogenicity symptoms for 7 days after each injection
- Change from baseline in safety laboratory measures
- Occurrence of AEs of all severities through the 4-week post-injection visit
- Occurrence at any time throughout the study of SAEs or new chronic medical conditions that require ongoing medical management.

6.3.2 Secondary Endpoints: Immunogenicity

The principal immunogenicity endpoints will be assessed by detection of stem-specific responses to H10ssF-6473 at:

1. Group 1: Baseline (Week 0/Visit 02) and Week 2 (Visit 04) after vaccination, and

2. Groups 2A, 2B: Baseline (Week 0/Visit 02) and Week 2 (Visit 04) after the first vaccination, and Week 16 (Visit 07) and Week 18 (Visit 09) after the second vaccination

6.3.3 Exploratory Endpoints: Immunogenicity

Exploratory immunogenicity evaluations may include the detection of antibody by MSD or similar platform, microneutralization assay, and exploratory B and T cell assays.

6.3.4 Power Calculations for 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. Other sample size calculations for comparing the two vaccination groups on adverse experiences are similar to the calculations for immunogenicity (6.4.3).

The ability of the study to identify SAEs will be expressed in terms of the probability of observing a certain number of SAEs. Useful values are the minimum true rate such that the probability of observing at least one event is at least 90%, and the maximum true rate such that the probability of not observing any event is at least 90%.

For Group 1 (*dose-escalation*) within each group (n=5), there is a 90% chance to observe at least 1 SAE if the true rate is at least 0.37 and over 90% chance to observe no SAE if the true rate is less than 0.02.

For Groups 2A and 2B within each group (n=10), there is greater than a 90% chance to observe at least 1 SAE if the true rate is at least 0.21 and over a 90% chance to observe no SAE if the true rate is no more than 0.01.

For Groups 2A and 2B within each group (n=15), there is greater than a 90% chance to observe at least 1 SAE if the true rate is at least 0.145 and over a 90% chance to observe no SAE if the true rate is no more than 0.0069.

Probabilities of observing 0 or more than 1 SAE within each group are presented in

Table 3 for a range of possible true event rates and different sample sizes. These calculations provide a complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 4 gives the upper and lower bounds for 95% exact binomial confidence intervals of the true SAE rate at all possible numbers of events within each group (n=5 and n=10-15).

For Group 1 (n=5): If none of the 5 participants receiving the vaccines experience SAEs, the 95% exact 2-sided upper confidence bound for the SAE rate is 0.522.

For Group 2A and 2B (n=10 each): If none of the 20 participants receiving the vaccines experience SAEs, the 95% exact 2-sided upper confidence bound for the SAE rate is 0.168.

For Groups 2A and 2B (n=15 each): If none of the 30 participants receiving the vaccines experience SAEs, the 95% exact 2-sided upper confidence bound for the SAE rate is 0.116.

Table 3: Probability of Events for Different Safety and Immunogenicity Scenarios

	Group 1 (*dose-escalation*): N=5		Groups 2A and 2B: N=10		Groups 2A and 2B combined: N=20	
True Event Rate	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)
0.005	0.975	<0.001	0.951	0.001	0.905	0.004
0.010	0.951	0.001	0.904	0.004	0.818	0.017
0.020	0.904	0.004	0.817	0.016	0.668	0.060
0.035	0.837	0.011	0.700	0.046	0.490	0.154
	Group 1 (*dose-escalation*): N=5		Groups 2A and 2B: N=15		Groups 2A and 2B combined: N=30	
True Event Rate	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)
0.050	0.774	0.023	0.599	0.086	0.358	0.264
0.100	0.590	0.081	0.349	0.264	0.122	0.608
0.150	0.444	0.165	0.197	0.456	0.039	0.824
0.200	0.328	0.263	0.107	0.624	0.012	0.931
0.300	0.168	0.472	0.028	0.851	0.001	0.992
0.400	0.078	0.663	0.006	0.954	<0.001	0.999
0.500	0.031	0.812	0.001	0.989	<0.001	>0.999
	Group 1 (*dose-escalation*): N=5		Groups 2A and 2B: N=15		Groups 2A and 2B combined: N=30	
True Event Rate	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)	Pr (observing 0 event)	Pr (observing more than 1 event)
0.005	0.975	<0.001	0.928	0.003	0.860	0.010
0.010	0.951	0.001	0.860	0.010	0.740	0.036
0.020	0.904	0.004	0.739	0.035	0.545	0.121
0.035	0.837	0.011	0.586	0.095	0.343	0.283
0.050	0.774	0.023	0.463	0.171	0.215	0.446
0.100	0.590	0.081	0.206	0.451	0.042	0.816
0.150	0.444	0.165	0.087	0.681	0.008	0.952
0.200	0.328	0.263	0.035	0.833	0.001	0.989
0.300	0.168	0.472	0.005	0.965	<0.001	>0.999
0.400	0.078	0.663	<0.001	0.995	<0.001	>0.999
0.500	0.031	0.812	<0.001	>0.999	<0.001	>0.999

Table 4: 95% Confidence Intervals for the True Rate at All Possible Observed Rates within a Group (n=5 and n=10-15)

	Group 1: N=5 95% Confidence Interval				Groups 2A and 2B: N=20 95% Confidence Interval	
Observed Rate	Lower Bound	Upper Bound		Observed Rate	Lower Bound	Upper Bound
0/5	0.000	0.522		0/20	0.000	0.168
1/5	0.005	0.716		1/20	0.001	0.249
2/5	0.053	0.853		2/20	0.012	0.317
3/5	0.147	0.947		3/20	0.032	0.379

4/5	0.284	0.995		4/20	0.057	0.437
5/5	0.478	1.000		5/20	0.087	0.491
				6/20	0.119	0.543
				7/20	0.154	0.592
				8/20	0.191	0.639
				9/20	0.231	0.685
				10/20	0.272	0.728
				11/20	0.315	0.769
				12/20	0.361	0.809
				13/20	0.408	0.846
				14/20	0.457	0.881
				15/20	0.509	0.913
				16/20	0.563	0.943
				17/20	0.621	0.968
				18/20	0.683	0.988
				19/20	0.751	0.999
				20/20	0.832	1.000
	Group 1: N=5 95% Confidence Interval				Groups 2A and 2B: N=20 95% Confidence Interval	
Observed Rate	Lower Bound	Upper Bound		Observed Rate	Lower Bound	Upper Bound
0/5	0.000	0.522		0/30	0.000	0.116
1/5	0.005	0.716		1/30	0.001	0.172
	Group 1: N=5 95% Confidence Interval				Groups 2A and 2B: N=20 95% Confidence Interval	
Observed Rate	Lower Bound	Upper Bound		Observed Rate	Lower Bound	Upper Bound
2/5	0.053	0.853		2/30	0.008	0.221
3/5	0.147	0.947		3/30	0.021	0.265
4/5	0.284	0.995		4/30	0.038	0.307
5/5	0.478	1.000		5/30	0.056	0.347
				6/30	0.077	0.386
				7/30	0.099	0.423
				8/30	0.123	0.459
				9/30	0.147	0.494
				10/30	0.173	0.528
				11/30	0.199	0.561
				12/30	0.227	0.594
				13/30	0.255	0.626
				14/30	0.283	0.657
				15/30	0.313	0687
				16/30	0.343	0.717
				17/30	0.374	0.745
				18/30	0.406	0.773
				19/30	0.439	0.801
				20/30	0.472	0.827
				21/30	0.506	0.853
				22/30	0.541	0.877
				23/30	0.577	0.901
				24/30	0.614	0.923
				25/30	0.653	0.944

				26/30	0.693	0.962
				27/30	0.735	0.979
				28/30	0.779	0.992
				29/30	0.828	0.999
				30/30	0.884	1.000

6.3.5 Power Calculations for Immunogenicity

Table 3 gives the probabilities of observing 0 or more than 1 response over a range of underlying response rates and different sample sizes.

Table 4 is applicable to the immunogenic response rates and gives the exact 95% confidence interval of the true response rate over possible number of responses out of the 5 and 10 subjects.

6.3.6 Power for Comparison

As exploratory analyses, we will conduct comparisons between Groups 1 and 2 for possible differences in immunogenicity; a simple comparison on the positive response rate will be used.

Tables Table 5 **and**

Table 6 give the power of Fisher's exact test to compare the groups over a range of possible response rates. It indicates that a comparison between the groups is not powered by the study design with 5 and 20 or 30 samples for Group 1 and Group 2, respectively, unless the difference between rates is very large.

In addition, we will conduct comparisons between groups 2A and 2B.

Tables

Table 7 **and 8** give the power of Fisher's exact test to compare the groups over a range of possible response rates. It indicates that a comparison between these groups is not powered by the study design with 10 (15) samples per group unless the difference among rates is very large.

Table 5: Power to Detect Difference in Response Rates between Groups 1 and 2 by Fisher's Exact Test, where Group 1 is of size 5 and Group 2 is of size 20.

		Group 2 n=20									
		0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
Group 1 n=5	0.1	2	0	1	8	25	48	71	87	97	100
	0.2	7	2	1	4	14	30	49	70	89	99
	0.3	16	6	2	3	8	17	32	52	74	97
	0.4	28	12	5	3	4	9	20	35	60	92
	0.5	43	22	10	5	4	5	10	23	43	81

	0.6	60	35	19	9	4	3	5	12	29	66
	0.7	75	53	32	17	8	3	2	6	16	47
	0.8	88	71	48	30	14	4	1	2	7	27
	0.9	97	87	71	49	25	7	1	0	2	8
	1	100	100	95	75	41	13	2	0	0	0

Table 6: Power to Detect Difference in Response Rates between Groups 1 and 2 by Fisher's Exact Test, where Group 1 is of size 5 and Group 2 is of size 30

		Group 2 n=30									
		0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
Group 1 n=5	0.1	2	0	0	5	25	52	72	90	98	100
	0.2	9	2	1	3	14	31	50	73	92	99
	0.3	19	6	2	2	7	17	33	56	81	97
	0.4	34	14	5	2	4	9	20	39	66	92
	0.5	50	26	10	4	3	5	11	25	49	82
	0.6	66	39	20	9	4	3	5	14	35	67
	0.7	81	56	33	18	7	2	2	6	19	48
	0.8	91	74	51	31	15	3	1	2	8	26
	0.9	98	89	72	52	26	6	0	0	2	8
	1	100	100	98	83	43	10	1	0	0	0

Table 7: Power to Detect Difference in Response Rates between Groups 2A and 2B by Fisher's Exact Test, where both groups are of size 10

		Group 2 n=10									
		0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
Group 1 n=10	0.1	0	1	6	15	29	47	66	85	96	100
	0.2	1	1	2	6	13	26	42	64	84	99
	0.3	5	2	1	2	5	12	25	42	67	95
	0.4	15	6	2	1	2	5	12	25	49	83
	0.5	30	13	6	2	1	2	5	14	29	62
	0.6	47	25	12	6	2	1	2	6	15	38
	0.7	66	42	24	13	6	2	1	2	5	15
	0.8	84	63	43	25	13	6	2	1	1	3
	0.9	96	84	67	47	29	15	5	1	0	0
	1	100	99	95	84	62	36	15	4	0	0

Table 8: Power to Detect Difference in Response Rates between Groups 2A and 2B by Fisher's Exact Test, where both groups are of size 15

		Group 2 n=15									
		0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
Group 1 n=15	0.1	1	4	15	32	53	74	89	98	100	100
	0.2	4	2	4	11	25	45	68	87	98	100
	0.3	15	4	2	3	10	22	44	68	89	100
	0.4	32	11	4	2	3	10	23	45	75	99
	0.5	54	25	9	3	2	3	9	25	53	94
	0.6	74	45	23	10	3	1	4	11	33	78
	0.7	89	68	43	23	10	3	2	4	15	49
	0.8	98	87	68	44	24	11	4	2	4	16
	0.9	100	97	89	74	54	32	15	4	1	1
	1	100	100	100	99	94	78	49	17	1	0

6.4 STATISTICAL ANALYSIS

All statistical analyses will be performed using Statistical Analysis System (SAS) (SAS Institute, Cary, NC), R, or S-Plus statistical software. No formal multiple comparison adjustments will be employed for safety endpoints or secondary endpoints.

6.4.1 Analysis Variables

The analysis variables consist of baseline variables and safety variables for primary and secondary objective analyses.

6.4.2 Baseline Demographics

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

6.4.3 Safety Analysis

6.4.3.1 Solicited Reactogenicity

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

6.4.3.2 Adverse Events

AEs are coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentages of participants experiencing each specific AE will be tabulated by severity and relationship to treatment. For the calculations in these tables, each participant's AE will be counted once under the maximum severity or strongest recorded causal relationship to treatment.

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

6.4.3.3 Local Laboratory Values

Boxplots, violin plots, or beeswarm plots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each plot 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.

6.4.4 Immunogenicity Analysis

The statistical analysis for immunogenicity will employ the intent-to-treat principle whereby all data from enrolled subjects will be analyzed according to the group assignment. However, if during immune assessment on stored samples, a subject is found to have a positive antibody response at baseline, the vaccine immune responses assessment for these subjects will not be included in the final immunogenicity analysis. If needed, a per-protocol analysis will be performed as secondary analysis where subjects will be analyzed according to their actual vaccination scheme if it is different from the assigned or up to the last visit in the study if there are early dropouts. The study is not designed to power the comparison in immune responses between vaccine dosages.

If assay data are qualitative (i.e., positive or negative) then analyses will be performed by tabulating the frequency of positive response for each assay at each time point that an assessment is performed. Binomial response rates will be presented with their corresponding exact 95% confidence interval estimates.

Some immunologic assays have underlying continuous or count-type readout that is often dichotomized into responder/non-responder categories. For these assays, graphical and tabular summaries of the underlying distributions will be made. These summaries may be performed on transformed data (e.g., log transformation) for ease of interpretation.

6.4.5 Missing Data

Missing responses will be assumed to be missing completely at random. Analyses will include all samples available at each study time point. Based on experience from previous trials, we expect missing data to be rare. Regardless, in the event of missing data, we will report the occurrence and extent of missingness. We will also provide plausible explanations for the missingness mechanism, should such information be available.

6.4.6 Interim Analyses

Safety Reviews: The PSRT will review safety data routinely throughout the study. The study will utilize both electronic database features and reviews by designated safety review personnel to identify in a timely manner if any of the safety pause rules of the study are met.

Immunogenicity Review: Analyses of immunogenicity may be performed when pseudo-neutralization assay of samples collected 2 weeks after each vaccination are conducted. This may occur prior to completion of safety follow-up visits or collection of data for secondary and exploratory immunogenicity endpoints. Such an analysis would constitute the final analysis for the primary immunogenicity endpoint, so sample size adjustments are not required. Reports providing results by study group may be provided to VRC solely for the purpose of informing decisions related to future trials in a timely manner. The results should in no way influence the

conduct of the VRC 323 trial in terms of early termination or later safety or immunogenicity endpoint assessments. Analyses of secondary and exploratory immunogenicity assays may also be performed as data become available.

7. PHARMACY AND VACCINE ADMINISTRATION PROCEDURES

The study groups and study agent dosing schedule are shown in **Table 2** in **Section 4.6**.

7.1 STUDY PRODUCT

The study includes one investigational vaccine described as follows:

- VRC-FLUNPF0103-00-VP is a sterile aqueous buffered solution aseptically filled into 3 mL single-dose glass vials. Each vial contains 0.7 ± 0.1 mL at a concentration of 180 ± 36 mcg/mL in formulation buffer composed of 20 mM sodium phosphate, pH 7.2, 100 mM sodium chloride, 5% w/v sucrose, 0.01% w/v Pluronic F-68 (aka Poloxamer 188). Vials are intended for single use only and do not contain preservative. Vials must not be refrozen or reused after thawing.

The diluent, VRC-PBSPLA043-00-VP (PBS), as described in Drug Master File 20054, will be used to prepare the 20 mcg of VRC-FLUNPF0103-00-VP for Group 1 subjects. PBS is a sterile aqueous buffered solution aseptically filled into 3 mL single-dose glass vials. Each vial contains 1.5 mL ± 0.1 mL volume of diluent composed of phosphate buffered saline at pH 7.2. Vials are intended for single use only and do not contain preservative. Vials must not be refrozen or reused after thawing.

7.2 STUDY PRODUCT PRESENTATION AND STORAGE

7.2.1 Study Product Labels

At the time of study product delivery to the pharmacy, labels on study products VRC-FLUNPF0103-00-VP and VRC-PBSPLA043-00-VP will have specific product information (e.g., product description, VRC product number, lot number, fill volume, concentration, fill date, storage condition). Labels will contain an Investigational Use Statement ("Limited by Federal Law to Investigational Use") and manufacturer information.

7.2.2 Study Product Storage

VRC-FLUNPF0103-00-VP (H10ssF-6473): Vials will be shipped within the recommended temperature range using appropriate shipping configurations to the study pharmacist or designee. Vials of vaccine are stored until use at -35°C to -15°C in a qualified, continuously monitored, temperature-controlled freezer. 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.

VRC-PBSPLA043-00-VP (PBS): Vials are stored until use at the target temperature of -35°C to -15°C in a qualified, continuously monitored, temperature-controlled freezer.

7.2.3 Study Product Handling Information

H10ssF-6473 and PBS (diluent) are not hazardous chemicals under U.S. OSHA Hazard Communication (29 CFR 1910.1200) and the Department of Transportation (49 CRF 172.101) standards. Although it has not been completely characterized, this vaccine is not known to cause significant acute health effects by casual contact, and there are no occupational exposure limits established by OSHA, ACGIH, or NIOSH. Handling of the study product should follow general laboratory safety practices to prevent unintended exposure. Protective gloves, safety glasses, and a lab coat should be worn.

In the event of a spill, procedures include use of proper personal protective equipment, physical containment with common absorbent materials, absorption of liquid with common absorbent materials, and disposal in appropriate closed containers. Spills on skin or splashes in eyes should be flushed with running water for at least 15 minutes. Accidental ingestion response should include washing the mouth and seeking medical attention. Waste materials should be disposed of in accordance with standard institutional procedures.

For administration of the prepared product in the clinical setting, the clinical staff should practice universal precautions and dispose of the used needles, syringes and IV bags in keeping with the required practices for handling sharps in the medical facility.

7.2.4 Temperature Excursions

If deviations in storage temperature occur from the normal allowance for the pharmacy freezer, the site pharmacist or designee must report the storage temperature excursion promptly to the PI and IND Sponsor. 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 if continued clinical use of the product is acceptable.

In the case of storage or shipping/handling temperature excursions outside of the normal allowance for the storage device, the following procedure is to be followed:

1. Quarantine the affected product in a separate area. If the excursion results in thawed material, it must not be refrozen. Thawed vials must be quarantined at 5°C ± 3°C.
2. Report the excursion to the IND sponsor's authorized representative (SAR) or designee, any other parties required by site procedures, and via email to VRCProductinquiries@nih.gov. Do not use until the IND SAR or designee informs the site pharmacist whether continued clinical use of the product is acceptable.
3. Inquiries sent to VRCProductinquiries@nih.gov will prompt an automatic email reply to the notifier that includes the Clinical Excursion Reporting Form (CERF) as an attachment.
4. Fill out the CERF as completely as possible, either electronically or manually followed by scanning to generate an electronic copy.
5. Email the completed form and relevant attachments (e.g. temperature charts) to VRCProductinquiries@nih.gov, replying to the previous email.
6. After receipt and evaluation of the reported information, the Sponsor or manufacturer's designee will notify the site pharmacist whether continued clinical use of the product is acceptable.

7.3 PREPARATION OF STUDY PRODUCT FOR ADMINISTRATION

This section describes how the site pharmacist will prepare study injections. Refer to the group assignment for the study subject to prepare the correct dose. All vials are intended for single use.

For all study groups, the prepared syringe must be labeled with the subject identifier and the date and time after which the preparation may not be used. Filled syringes should be kept at room temperature and out of direct sunlight until product administration. All injections must be administered within 4 hours after removing the vaccine vial from the freezer.

The study product appearance specification is clear, colorless, no turbidity, some small white or translucent particles may be present. If some small white or translucent particles are present, the clinician may proceed with the product administration. The appearance specification for PBS is clear, colorless liquid.

7.3.1 Preparation of VRC-FLUNPF0103-00-VP for Administration

Subjects will receive the vaccine via needle and syringe injection. The following instructions apply for VRC-FLUNPF0103-00-VP preparation.

Group 1 (*20 mcg*):

A. Thaw 1 vial of H10ssF-6473 at room temperature (15º to 30º C) until all ice crystals have melted. Swirl gently to mix.

B. Thaw 1 vial of PBS diluent at room temperature (15º to 30º C) until all ice crystals have melted. Swirl gently to mix.

C. Withdraw 0.11 mL of H10ssF-6473 into the syringe using an 18-gauge needle; discard needle.

D. Withdraw 0.2 mL of PBS into the same syringe using an 18-gauge needle; discard needle and cap the syringe for transport.

E. Invert syringe 5x to mix.

Groups 2A and 2B (*60 mcg*):

1. Thaw 1 vial of H10ssF-6473 at room temperature (15º to 30º C) until all ice crystals have melted. Swirl gently to mix.
2. Withdraw 0.33 mL of H10ssF-6473 into the syringe using an 18-gauge needle; discard needle and cap the syringe for transport.

7.3.2 Administration of Injections

The study product appearance is clear, colorless, no turbidity; some small white or translucent particles may be present. If some small white or translucent particles are present, the clinician may proceed with the product administration.

Clinician instructions on how to select an arm and administer an injection are in 4.3.8. Subjects will receive the vaccine using needle and syringe injection in either deltoid. Clinicians will choose the appropriate needle size for each subject (either a 21-gauge 1-inch length or a 22-gauge 1.5 inch length). Product labeling verification and IM injection procedures will be performed consistent with institutional policies and standard procedures.

7.4 STUDY PRODUCT ACCOUNTABILITY

7.4.1 Documentation

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

7.4.2 Disposition

Empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with the institutional or pharmacy policy. Partially used vials will not be administered to other subjects or used for in vitro experimental studies.

Any unopened vials that remain at the end of the study may be returned to the VRC or discarded at the discretion of the sponsor in accordance with policies that apply to investigational agents. Vials will be disposed of in accordance with institutional or pharmacy policy.

8. HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research study will be conducted in compliance with the protocol, International Council for Harmonisation Good Clinical Practices (ICH-GCP) guidance, and all applicable regulatory requirements.

8.1 INSTITUTIONAL REVIEW BOARD

A copy of the protocol, ICF, other written subject-facing information, and any advertising material will be submitted to the IRB for written approval prior to use.

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 research events that occur on study as described in 5.5.

The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

8.2 INFORMED CONSENT

The study informed consent form (ICF) is provided as a separate document and describes the investigational product to be used and all aspects involved in protocol participation.

The PI or designee is responsible for obtaining written informed consent from the subject after adequate explanation of the aims, methods, anticipated risks and benefits of the study and before any protocol-specific procedures or study product is administered. The AoU must be completed before the study ICF is signed.

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 the person who conducted the informed consent discussion. The signed ICF will be retained in the medical chart and a copy of the ICF will be provided to the subject.

8.3 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the investigators, the Investigational New Drug (IND) and regulatory authorities as appropriate. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and Sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB, Office for Human Research Protections (OHRP), and/or FDA.

8.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the Sponsor(s) and their representatives. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored by The Emmes Company, LLC (Rockville, MD), the Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the

clinical site and by Emmes research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

8.5 RISKS AND BENEFITS ASSESSMENT

8.5.1 Risks of VRC-FLUNPF0103-00-VP

This is the first study in humans of the investigational vaccine, VRC-FLUNPF0103-00-VP, and therefore risks are unknown at the time of study start.

The following signs and symptoms have been associated with administration of similar vaccines as discussed in Sections 1.3.2**Error! Reference source not found.** and 1.3.3. Subjects have reported mild local pain/tenderness at the injection site, mild headache, mild malaise, mild to moderate myalgia, mild chills, mild nausea, and mild joint pain. One subject also reported mild abnormal dreams. A small subset of subjects experienced mild to moderate laboratory changes of leukocytosis, increased blood alkaline phosphatase, and leukopenia. In addition, no SAEs have occurred which were determined to be related to the similar study products assessed in previous studies.

Potential side effects resulting from IM injection include stinging, pruritis, 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, 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 other unknown side effects.

8.5.2 Risks of Specimen Collections

- *Blood drawing*: The risks of blood sample collection are minimal and consist of mild discomfort at the sample collection site. The procedure may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.
- *Apheresis*: The procedure may cause pain, bruising, and discomfort in the arms where the needles are placed. It may also cause chills, nausea, heartburn, mild muscle cramps and tingling sensation around the mouth or in the fingers, however this can usually be relieved by slowing or temporarily interrupting the apheresis procedure or taking a calcium containing antacid, such as Tums®. Other possible side effects are anxiety, vomiting and lightheadedness. Temporary lowering of the blood pressure may develop.

There is the rare possibility of infection, fainting or seizure. Very rarely a nerve problem at the needle placement site may occur. Also, very rarely, a machine malfunction may occur, resulting in the loss of about one unit of blood. There may be additional risks of apheresis that are unknown at this time.

- *Mucosa sampling*: Collection of samples by nasopharyngeal or oral swabs rubbed over the mucosal surfaces may cause momentary discomfort and, in some cases, minor bleeding.

8.5.3 Risks of Study Vaccine on the fetus or nursing infant

We do not know the possible effects of the study vaccine on the fetus or nursing infant. Women of reproductive potential will be required to agree to use an effective method of birth control beginning 21 days prior to enrollment and continuing through end of study.

Because this is a research study, women of reproductive potential will be tested for pregnancy prior to administration of each study injection and asked to notify the site immediately upon learning of a pregnancy during this study. In the case of pregnancy, subjects will no longer receive additional vaccine but will continue to be followed for safety. Research sample collections will be discontinued for pregnant women. The subject will be contacted to ask about the outcome of a pregnancy that begins during the study.

8.5.4 Risks of New Diagnoses

It is possible that the standard medical tests performed as part of this research protocol will result in new diagnoses. Depending upon 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, will be forwarded to the subject's primary health care provider for further workup and management.

8.5.5 Potential 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 H10 (or universal) influenza virus vaccine. The investigational vaccine is not expected to provide protection from influenza.

8.5.6 Assessment of Potential Risks and Benefits

This healthy volunteer trial to evaluate the safety and immunogenicity of H10ssF-6473 was reviewed using the VRC Risk Management Plan. Potential risks, acceptance of risks, and mitigation strategies are available in the VRC 323 Risk Register.

8.6 PLAN FOR USE AND STORAGE OF BIOLOGICAL SAMPLES

The plan for use and storage of biological samples from this protocol is as outlined in the following sections.

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.

Stored samples may be used later to further evaluate immune responses and to elucidate genetic factors associated with immune response. No personal identifiable information will be shared since the results will only be shared with a code.

Other optional analysis, including proteome, lipidome, metabolome, and exosome may be done on collected specimens to evaluate some proteins, lipids, metabolites, and low molecular weight molecules involved in the immune response to vaccination.

8.6.2 Storage and Tracking of Blood Samples and Other Specimens

All of the stored study research samples are labeled by a code that only the site can link to the subject. Samples are stored at the VIP, Gaithersburg, MD or VRC laboratories in Building 40, Bethesda, MD, which are both secure facilities with limited access. Data will be kept in password-protected computers. Only investigators or their 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. IRB approval must be sought prior to any sharing of samples. Any clinical information shared about those samples would similarly require prior IRB approval. The research use of stored, unlinked or unidentified samples may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research, which is authorized to determine whether a research activity is exempt.

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 IRB and study sponsor approval, it may be either destroyed or transferred to another repository.

8.6.4 Loss or Destruction of Samples, Specimens or Data

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 SAFETY OVERSIGHT

8.7.1 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. The VRC designated Safety Officer for the day conducts a daily safety review of clinical data per VRC Standard Operating Procedures. The PSRT, comprised of the PI, Associate Investigators, Study Coordinator, Protocol Specialists, and other Study Clinicians, will review the summary study safety data reports on a weekly basis through 4 weeks after the last subject receives the final study injection. After this time, the PSRT will monitor the safety data reports on a monthly basis through completion of the last study visit.

9. ADMINISTRATIVE AND OPERATIONAL OBLIGATIONS

9.1 PROTOCOL AMENDMENTS AND STUDY TERMINATION

Protocol amendments must be made only with prior approval of the IND Sponsor and with agreement from the PI and MO. All study amendments will be submitted to the IRB for approval.

The IND Sponsor, the IRB, OHRP, the PI, Protocol Chairs, and/or the FDA reserve the right to terminate the study. The PI will notify the IRB in writing of the study's completion or early termination.

9.2 STUDY DOCUMENTATION AND STORAGE

The PI will delegate the study responsibilities to the study team, and a list of appropriately-qualified persons to whom trial duties have been delegated will be maintained.

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, and correspondence. Long-term storage of source documents may be in the form of electronic files.

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

1. Subject files containing completed informed consent forms and supporting copies of source documentation.
2. Study files containing the protocol with all amendments, IBs, copies of all correspondence with the IRB.

In addition, all original source documentation must be maintained and be 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 up to three years after marketing approval or refusal (21 CFR 312.62). If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued and the FDA is notified. The HHS protection of human subjects'

regulations require that institutions retain records of IRB/EC activities and documentation of informed consent of subjects for at least 3 years after study completion (45 CFR 46).

No study document should be destroyed without prior written agreement between the VRC and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, they must notify the VRC in writing of the new responsible person and/or the new location.

9.3 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

Monitoring for this study will be performed by a designated contract research organization (CRO), Technical Resources International, Inc. Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

9.4 DATA COLLECTION AND DATA SHARING

9.4.1 Data Collection

Clinical research data will be collected in a secure electronic web-based clinical data management system (CDMS) through a CRO, The Emmes Company, LLC (Rockville, MD). Extracted data without patient identifiers will be sent to the Protocol Statistician for statistical analysis.

9.4.2 Source Documents

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6(R2) GCP, applicable regulations, 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, laboratory reports, pharmacy records and other research records maintained for the clinical trial.

9.4.3 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 or within 1 year of the primary completion date.

9.5 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. The VEC's Quality Management Plan will be used to perform quality management for this trial.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

The monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

9.6 LANGUAGE

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

9.7 RESEARCH-RELATED INJURIES

The NIH CC will provide short-term medical care for any injury resulting from participation in this research. In general, the NIH, the NIH CC, or the U.S. Federal Government will provide no long-term medical care or financial compensation for research-related injuries.

10. REFERENCES

1. WHO. *Influenza (Seasonal) Fact Sheet*. 2009; Available from: http://www.who.int/mediacentre/factsheets/fs211/en/index.html.
2. *Disease Burden of Influenza*. February 19, 2019; Available from: https://www.cdc.gov/flu/about/burden/index.html.
3. Meltzer, M.I., N.J. Cox, and K. Fukuda, *The economic impact of pandemic influenza in the United States: priorities for intervention.* Emerg Infect Dis, 1999. **5**(5): p. 659-71.
4. Luke, C. and K. Subbarao, *Vaccines for pandemic influenza.* Emerging Infectious Diseases, 2006. **12**(1): p. 66-72.
5. CDC. *Types of Influenza Viruses*. 2014 4/15/2014]; Available from: http://www.cdc.gov/flu/about/viruses/types.htm.
6. Medina, R.A. and A. Garcia-Sastre, *Influenza A viruses: new research developments.* Nat Rev Microbiol, 2011. **9**(8): p. 590-603.
7. Taubenberger, J.K. and J.C. Kash, *Influenza virus evolution, host adaptation, and pandemic formation.* Cell Host Microbe, 2010. **7**(6): p. 440-51.
8. D C Wiley, a. and J.J. Skehel, *The Structure and Function of the Hemagglutinin Membrane Glycoprotein of Influenza Virus.* Annual Review of Biochemistry, 1987. **56**(1): p. 365-394.
9. Ekiert, D.C., et al., *Antibody recognition of a highly conserved influenza virus epitope.* Science, 2009. **324**(5924): p. 246-51.
10. Throsby, M., et al., *Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells.* PLoS One, 2008. **3**(12): p. e3942.
11. Corti, D., et al., *A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins.* Science, 2011. **333**(6044): p. 850-6.
12. Harris, A.K., et al., *Structure and accessibility of HA trimers on intact 2009 H1N1 pandemic influenza virus to stem region-specific neutralizing antibodies.* Proc Natl Acad Sci U S A, 2013. **110**(12): p. 4592-7.
13. Tripp, R.A. and S.M. Tompkins, *Virus-vectored influenza virus vaccines.* Viruses, 2014. **6**(8): p. 3055-79.
14. CDC. *Vaccine Effectiveness - How Well Does the Flu Vaccine Work?* 2016 11/14/2016; Available from: https://www.cdc.gov/flu/about/qa/vaccineeffect.htm.
15. Lambert, L.C. and A.S. Fauci, *Influenza vaccines for the future.* N Engl J Med, 2010. **363**(21): p. 2036-44.
16. Krammer, F. and P. Palese, *Advances in the development of influenza virus vaccines.* Nat Rev Drug Discov, 2015. **14**(3): p. 167-82.
17. Neuzil, K.M. and R.A. Bright, *Influenza vaccine manufacture: keeping up with change.* J Infect Dis, 2009. **200**(6): p. 835-7.

18. Yassine, H.M., et al., *Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection.* Nat Med, 2015. **21**(9): p. 1065-70.

19. Corbett, K.S., et al., *Design of Nanoparticulate Group 2 Influenza Virus Hemagglutinin Stem Antigens That Activate Unmutated Ancestor B Cell Receptors of Broadly Neutralizing Antibody Lineages.* MBio, 2019. **10**(1).

20. Bai, L., et al., *Genome-wide comparison of ferritin family from Archaea, Bacteria, Eukarya, and Viruses: its distribution, characteristic motif, and phylogenetic relationship.* Naturwissenschaften, 2015. **102**(9-10): p. 64.

21. Kanekiyo, M., et al., *Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies.* Nature, 2013. **499**(7456): p. 102-6.

22. Yamashita, I., K. Iwahori, and S. Kumagai, *Ferritin in the field of nanodevices.* Biochim Biophys Acta, 2010. **1800**(8): p. 846-57.

23. Zhao, L., et al., *Nanoparticle vaccines.* Vaccine, 2014. **32**(3): p. 327-37.

24. Krammer, F., P. Palese, and J. Steel, *Advances in universal influenza virus vaccine design and antibody mediated therapies based on conserved regions of the hemagglutinin.* Curr Top Microbiol Immunol, 2015. **386**: p. 301-21.

25. Gaudinski, M.R., et al., *Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults.* PLoS Med, 2018. **15**(1): p. e1002493.

APPENDIX I: SCHEDULE OF EVALUATIONS

		VRC 500	VRC 323 Schedule of Evaluations: Group 1									
Visit Number		*01	02	02A	03	04	05	06	07	09	12	13
Week of Study		-4 to 0	W0	W1	W1	W2	W4	W12	W16	W18	W28	W40
Day of Study		-56 to 0	[1]D0	D1	D6	D14	D28	D84	D112	D126	D196	D280
Clinical	Tube											
*VRC 500 Screening Consent		X										
VRC 323 AoU; Consent			X									
[2] Physical exam for eligibility, height /weight/ vitals at screening; vital signs and targeted exam (as needed) other visits.		X	X		X	X	X	X	X	X	X	X
Medical history targeted to eligibility at screening; then interim medical history (as needed)		X	X		X	X	X	X	X	X	X	X
[3] Study Product Administration: **Group 1**			**X**									
Phone evaluation (clinic visit as needed)				X								
Begin diary card			X									
[4] Pregnancy test: urine or serum		X	X				X					X
[4] Pregnancy prevention counseling/ Reproductive Information Form		X	X				X					X
CBC with differential	EDTA	3	3			3	3					3
Iron and serum ferritin		X					X					
Total bilirubin, AST, ALT, and ALP	GLT	4	4			4	4					4
Creatinine		X	X			X						
HIV (other tests, if needed)	EDTA	3										
[5] SARS-CoV-2 PCR, nasopharyngeal swab		X										
Research Samples												
H. pylori ferritin antibody and human ferritin antibody	SST	X					X					
Serum	SST	32	16		16	16	16	16	16	16	16	16
PBMC and plasma	EDTA	80	80		[6]80	80	80	80	80	40	80	80
Daily Volume (mL)		122	103	0	96	103	103	96	96	56	96	103
Max. Cumulative Volume (mL)		122	225	225	321	424	527	623	719	775	871	974

* VRC 500: Screening evaluations must be no more than 56 days prior to Day 0 to be used for eligibility (negative pregnancy test from Day 0 must be used for eligibility). If clinical assessment on Day 0 suggests significant changes have occurred since screening, then physical exam & laboratory studies done on Day 0 may be used for eligibility. Research blood samples will be collected anytime during screening through enrollment and are not subject to the "56-day prior to enrollment" restriction.

1 Day 0=day of enrollment and first vaccination. Day 0 evaluations prior to first injection are the baseline for assessing adverse events subsequently.

2 Screening visit includes physical exam with vital signs. At other visits, physical exam is done if indicated. Otherwise only blood pressure (BP), pulse, temperature, and respiration are required.

3 Product Administration: **Group 1** will receive 20 mcg IM of H10ssF-6473 at Day 0. Complete post vaccination evaluations (BP, pulse, temperature, respiration and injection site assessment) at **30 minutes** or longer after injection.

4 Negative pregnancy test results must be confirmed for women of reproductive potential prior to each study injection.

5 A nasopharyngeal swab for the SARS-CoV-2 PCR should be collected no more than 4 days prior to product administration.

6 Visit 03: Two tubes of PBMC collected in EDTA tubes will be sent to Building 40, while the remainder of the blood collected will be sent to VIP.

Visit windows: Schedule visits 02A - 13 with respect to Day 0 per the following visit windows: Visit 02A (+1 day). Visit 03 (±1 day). Visits 04, 05, 09 (±2 days). Visit 07 (± 7 days). Visits 06, 12, 13 (±14 days).

Of note: Schedule for Group 1 does not have Visit 10 and Visit 11.

		VRC 500	VRC 323 Schedule of Evaluations: Groups 2A and 2B														
Visit Number		*01	02	02A	03	04	05	06	06C	07	07A	08	09	10	11	12	13
Week of Study		-4 to 0	W0	W1	W1	W2	W4	W12	W16	W16	W17	W17	W18	W20	W22	W28	W40
Day of Study		-56 to 0	[1]D0	D1	D6	D14	D28	D84	D108	D112	D113	D118	D126	D140	D154	D196	D280
Clinical	Tube																
*VRC 500 Screening Consent		X															
VRC 323 AoU; Consent			X														
[2] Physical exam for eligibility, height /weight/ vitals at screening; vital signs and targeted exam (as needed) other visits.		X	X		X	X	X	X	X	X		X	X	X		X	X
Medical history targeted to eligibility at screening; then interim medical history (as needed)		X	X		X	X	X	X	X	X		X	X	X		X	X
[3] Study Product Administration: **Groups 2A and 2B**			**X**							**X**							
Phone evaluation (clinic visit as needed)				X							X						
Begin diary card			X														
[4] Pregnancy test: urine or serum		X	X				X			X			[8][X]	X			X
[4] Pregnancy prevention counseling/ Reproductive Information Form		X	X				X			X			[8][X]	X			X
CBC with differential	EDTA	3	3			3	3			3				3			3
Iron and serum ferritin		X								X				X			
Total bilirubin, AST, ALT, and ALP	GLT	4	4			4	4			4				4			4
Creatinine		X	X			X								X			
HIV (other tests, if needed)	EDTA	3															
[5] SARS-CoV-2 PCR, nasopharyngeal swab		X							X								
Research Samples																	
Oral Mucosal sample collection for antibody analysis			X				X							X			
H. pylori ferritin antibody and human ferritin antibody	SST	X					X							X			
Serum	SST	32	16		16	16	16	16		16		16	16	16	56	16	16
PBMC and plasma	EDTA	80	80		[6]80	80	80	80		80		80	[7]120 or Apheresis (6)	80		80	80
Daily Volume (mL)		122	103	0	96	103	103	96	0	103	0	96	136	103	56	96	103
Max. Cumulative Volume (mL)		122	225	225	321	424	527	623	623	726	726	822	958	1,061	1,117	1,213	1,316

* VRC 500: Screening evaluations must be no more than 56 days prior to Day 0 to be used for eligibility (pregnancy test from Day 0 must be used for eligibility). If clinical assessment on Day 0 suggests significant changes have occurred since screening, then physical exam & laboratory studies done on Day 0 are used for eligibility. Research blood samples will be collected anytime during screening through enrollment and are not subject to the "56-day prior to enrollment" restriction.
1 Day 0=day of enrollment and first vaccination. Day 0 evaluations prior to first injection are the baseline for assessing adverse events subsequently.
2 Screening visit includes physical exam with vital signs. At other visits, physical examination is done if indicated. Otherwise only blood pressure (BP), pulse, temperature, and respiration are required.
3 Product Administration: **Groups 2A** and **2B** will receive 60 mcg IM of H10ssF-6473 at Day 0 and Week 16. Complete post vaccination evaluations (BP, pulse, temperature, respiration and injection site assessment) at **30 minutes** or longer after each injection
4 Negative pregnancy test results must be confirmed for women of reproductive potential prior to each study injection and prior to apheresis.
5 A nasopharyngeal swab for the SARS-CoV-2 PCR should be collected no more than 4 days prior to each product administration.
6 Visit 03: Two EDTA tubes for PBMC collected will be sent to Building 40, while the remainder of the blood collected will be sent to VIP.
7 If optional Apheresis occurs, ONLY draw 16 mL in SST (DO NOT draw 120 ml in EDTA tubes).
8 For women of reproductive potential, pregnancy test must be negative within 72 hours prior to apheresis procedure.
Visit windows: Schedule visits 02A - 07 with respect to Day 0; schedule visits 07A - 13 with respect to Visit 07. The following visit windows apply: Visits 02A, 07A (+1 day). Visits 03, 08 (±1 day). Visits 04, 09 (±2 days). Visits 05, 10 (±2 days). Visit 06C (≤4 days). Visits 06, 07 (±14 days). Visit 11 (± 3 days). Visits 12, 13 (±14 days).

APPENDIX II: ASSESSMENT OF RELATIONSHIP TO VACCINE AND GRADING SEVERITY OF ADVERSE EVENTS

Assessment of Relationship of an Adverse Event to Study Vaccine:

The relationship between an 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 agent are related in time, and a direct association can be demonstrated.
- **Probably Related**. The AE and administration of study agent are reasonably related in time, and the AE is more likely explained by study agent than other causes.
- **Possibly Related**. The AE and administration of study agent are reasonably related in time, but the AE can be explained equally well by causes other than study agent.
- **Not Related**. The AE is clearly explained by another cause not related to the study product.

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

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

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.
- 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.
- Severity grading for Grade 4 local reaction to injectable product (Erythema/Redness and Induration/Swelling) refer to necrosis or exfoliative dermatitis "requiring medical attention."
- Bruising or skin lesion associated with study injection will be assessed using the same severity grading as for erythema/redness.

When not otherwise specified, 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 (Potentially 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
Modified from FDA Guidance - September 2007

- **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
[1,2]Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis requiring medical attention
[3] 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
[5]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

[4] Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
[6]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. In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
3. Bruising or skin lesion associated with study injection will be assessed using the same severity grading as for erythema/redness.
   Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
4. Subject should be at rest for all vital sign measurements.
5. Oral temperature; no recent hot or cold beverages or smoking.
6. 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
Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
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
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 the 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
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***	>1.5 – 3.0 x ULN	>3.0 –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	> 3.0 – 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

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4) ****
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	1.4 – 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.

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