Protocol Title: VRC 325 (000410): A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of Mosaic Quadrivalent Influenza Vaccine Compared with a Licensed Inactivated Seasonal QIV, In Healthy Adults

NCT: 04896086

Documents:

- IRB-approved Protocol (v5.0 04JAN2024) - Statistical Analysis Considerations located in Section 6 of the Protocol
- Final IRB-approved Main Informed Consent (v5.0 10JAN2024) IRB Approval/Document Date: 11JAN2024

**Version 5.0**
**January 4, 2024**

# Vaccine Research Center

## Protocol VRC 325

## (NIH 000410)

**TITLE: A PHASE I OPEN-LABEL CLINICAL TRIAL TO EVALUATE THE DOSE, SAFETY, TOLERABILITY AND IMMUNOGENICITY OF MOSAIC QUADRIVALENT INFLUENZA VACCINE WITH AND WITHOUT ADJUPLEX COMPARED WITH A LICENSED INACTIVATED SEASONAL QIV, IN HEALTHY ADULTS.**

**ABBREVIATED TITLE: FLUMOS-V1 IN HEALTHY ADULTS**

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

**IND 27330**

**Investigational Products: FLUMOS-V1 and Adjuplex**
**Manufacturer:**
Manufactured for VRC by Leidos Biomedical Research, Inc., Frederick, Maryland, US

NIH Principal Investigator: Maxwell Norris, MD, VRC/NIAID
[redacted]

# TABLE OF CONTENTS

## LIST OF TABLES

# ABBREVIATIONS

| Abbreviation | Term |
|---|---|
| AE | adverse event |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| AoU | Assessment of Understanding |
| BMI | body mass index |
| 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 |
| FDA | Food and Drug Administration |
| FluMos-v1 | Influenza Mosaic vaccine, version 1 |
| 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 |
| MDCK | Madin Darby Canine Kidney |
| 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 |
| QIV | quadrivalent influenza vaccine |
| RBS | receptor binding site |
| ssRNA | single-stranded ribonucleic acid |
| 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 |
| TIV | trivalent inactivated vaccine |
| ULN | upper limit of normal |
| UP | Unanticipated Problem |
| hVCMP | Vaccine Clinical Materials Program |
| VIP | Vaccine Immunology Program |
| VRC | Vaccine Research Center |
| WBC | white blood cell |
| WHO | World Health Organization |

# PRINCIPAL INVESTIGATOR PROTOCOL SIGNATURE PAGE

**VRC 325**: A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of Mosaic Quadrivalent Influenza Vaccine with And without Adjuplex Compared with a Licensed Inactivated Seasonal QIV, 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.

| | |
|---|---|
| Maxwell Norris, MD | VRC / Vaccine Evaluation Clinic |
| Name/Title of Principal | Investigator Study Site Name |
| _____ | _____ |
| Signature of Principal Investigator | Date |

# PRÉCIS

**Title:** **VRC 325**: A Phase I Open-Label Clinical Trial To Evaluate the Dose, Safety, Tolerability and Immunogenicity of Mosaic Quadrivalent Influenza Vaccine with and without Adjuplex Compared with a Licensed Inactivated Seasonal QIV, in Healthy Adults.

**Design:** This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of the mosaic quadrivalent influenza vaccine VRC-FLUMOS0111-00-VP (FluMos-v1). The hypotheses are that the FluMos-v1 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 alone or with adjuvant in healthy adults. Secondary objectives relate to immunogenicity of the investigational vaccine and dosing regimen compared with the licensed inactivated seasonal Flucelvax® quadrivalent influenza vaccine (QIV) in healthy adults.

**Study Products:** The investigational vaccine FluMos-v1 was developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID) and is composed of the following 4 influenza strains: Influenza A (H1: A/Idaho/07/2018 and H3: A/Perth/1008/2019) and Influenza B (Victoria lineage: B/Colorado/06/2017 and Yamagata lineage: B/Phuket/3073/2013. FluMos-v1 is supplied in a single-use vial at a concentration of 180 mcg/mL.

In Part A, FluMos-v1 was compared to licensed 2020-2021 QIV Flucelvax® developed by Seqirus, Inc. and composed of the following 4 influenza strains: Influenza A (H1: A/Hawaii/70/2019 (H1N1) pdm09-like virus and H3: A/Hong Kong/45/2019 (H3N2)-like virus) and Influenza B: Victoria lineage: B/Washington/02/2019-like virus and Yamagata lineage: B/Phuket/3073/2013-like virus).

In Part B, a higher dose of FluMos-v1 was tested that more closely matches the amount of each HA antigen in Flucelvax. The adjuvant Adjuplex® was added to FluMos-v1 to evaluate the potential for increased immunogenicity. All study enrollments and injections have been completed, and no further enrollments are planned in this study.

Adjuplex is provided as a sterile, pyrogen-free, homogeneous solution filled to 0.7 mL in 3-mL glass vials. Adjuplex is mixed with study products in the pharmacy during preparation prior to vaccination at a 20% dose by volume.

FluMos-v1, FluMos-V1 plus Adjuplex, and Flucelvax® are administered intramuscularly (IM) in the deltoid muscle via needle and syringe.

**Subjects:** Healthy adults between the ages of 18-50 years inclusive will be enrolled.

**Study Plan**: In Part A, the study evaluated the safety, tolerability, and immunogenicity of a single dose of FluMos-v1 vaccine alone in a dose-escalation design.

In Part B, the study evaluated the safety, tolerability, and immunogenicity of a single dose of FluMos-v1 vaccine with or without Adjuplex.

Group 6 and Part C are optional, and a decision was made not to enroll the optional groups 6-8 in the study. All study enrollments and injections have been completed for groups 1-5, and no further enrollments are planned in this study.

The protocol requires 1 vaccination visit, about 8 follow-up visits, and a telephone contact on the day after vaccination. 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.

The study schema is as follows:

| VRC 325 Vaccination Schema | | | |
|---|---|---|---|
| **Group** | **Subjects** | **Day 0** | **Product** |
| **Part A** | | | |
| 1A<br>1B[1] | **5** | 20 mcg | FluMos-v1 |
| 2A<br>2B[1] | **15** | 60 mcg | FluMos-v1 |
| 3A<br>3B[1] | **15** | 60 mcg | Flucelvax® |
| **Part B** | | | |
| 4A<br>4B[2] | **13** | 100 mcg | FluMos-v1 |
| 5A<br>5B[2] | **13** | 100 mcg | FluMos-v1 + Adjuplex (20% v/v) |
| 6A<br>6B[2] | **13** | [3]60 mcg | FluMos-v1 + Adjuplex (20% v/v) |
| **[3]Part C** | | | |
| 7A<br>7B[2] | **13** | [3]X mcg | FluMos-v1 |
| 8A<br>8B[2] | **13** | [3]X mcg | FluMos-v1 + Adjuplex (20% v/v) |
| **Total** | **100** [4] | [1]Includes subjects who received the 2020-2021 seasonal influenza vaccine.<br>[2]Includes subjects who received the 2021-2022 or 2022-2023 seasonal influenza vaccine.<br>[3]Group 6 and Part C of the study are optional, and the decision was made not to enroll groups 6-8.<br>[4]Study enrollment has been completed with 35 subjects enrolled in Part A (Groups 1-3) and 26 in Part B (Groups 4-5) of the study. | |

**Study Duration**: Subjects will be evaluated for 40 weeks following vaccine administration and through an influenza season.

## 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. Influenza Virus

Influenza virus causes seasonal epidemics and pandemics at irregular intervals that result in significant morbidity and mortality. According to the World Health Organization (WHO) the annual epidemics result in 3 to 5 million cases of severe illness and about 290,000 to 650,000 respiratory deaths [1]. The Center for Disease Control (CDC) estimates that during the 2019-2020 influenza season, there were 56 million illnesses, 26 million medical visits, 740,000 hospitalizations, and 62,000 deaths [2]. Domestically, influenza has a high impact on costs of medical care, loss of productivity, and deaths annually [3]. In the US, the projected impact of the short-term costs and long-term burden of seasonal influenza can be up to $26.8 ~ $87.1 billion a year [4].

Of the five genera of influenza circulating in nature, only influenza A and B are known to cause outbreaks in humans [5]. Influenza A and B are negative single-stranded ribonucleic acid (ssRNA) viruses, that belong to the family *Orthomyxoviridae*. Their genome consists of 8 RNA gene segments that encoded for structural and non-structural proteins [6]. The major surface glycoproteins are: hemagglutinin (HA, responsible for host cell binding and virus entry) and neuraminidase (NA, responsible for virus budding and release) [7].

For influenza A and B, the HA is the predominant viral antigen target for antibody neutralization [8, 9]. HA glycoprotein consists of a globular head domain (which is highly variant in sequence among HA subtypes) and a stem domain (which is highly conserved across HA subtypes) [10]. 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 [11-13]. 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 [14].

Influenza A viruses are classified based on the antigenicity of HA and NA [15]. 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 [16]. 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 outbreaks [5].

Influenza A 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 and through zoonosis). The emergence of new influenza A strains through continuous mutation and reassortment of circulating virus diminishes the effectiveness of annual influenza vaccines [17, 18].

Influenza B viruses are classified into two distinct phylogenetic lineages: B/Yamagata and B/Victoria [9, 15]. There are two major differences between Influenza A and B; the first is a limited antigenic drift observed in influenza B, and the second is that influenza B lacks known animal reservoirs [19]. However, influenza B has the capacity to mutate which potentially can increase its pathogenicity and emerge as a pandemic strain. Also, Influenza B is the most prominent circulating strain of influenza every four to five years, it has been described to have

significantly higher mortality rates compared to influenza A strains, and is more fatal in children; furthermore, antiviral resistance is another major obstacle in the treatment of influenza B [19].

The threat of influenza B has been recently recognized and acknowledged by the introduction of the quadrivalent vaccine that includes both lineages of influenza B. These vaccines significantly decrease rates of infection; however, its effectiveness is low in susceptible populations [19].

Seasonal vaccination against influenza that circulates in humans, remains the best available intervention to prevent morbidity and mortality caused by influenza A and B [20]. However, the protection provided by the seasonal influenza vaccines varies from season to season and depends on the similarity between the strains selected each year by the WHO and those in circulation.

## 1.2. Rationale For Development of VRC-FLUMOS0111-00-VP, FluMos-v1

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 [21, 22].

The current manufacturing processes for most of the FDA approved influenza vaccines are in embryonated hen's eggs (egg-based systems) requiring use of viruses adapted to grow to high titers in eggs [22]. In the US, a strain mismatch in the licensed egg-based vaccines has occurred in past influenza seasons [23-27], since the production process in eggs has shown to cause egg adaptation, which results in a vaccine with different influenza strains antigenically from the prototype strains selected each year by the WHO [22, 28]. This manufacturing process takes about 6 months and can lead to lower yields and significant lag times due to virus strain identification [29-31]. Also, the capacity estimated in 2017 of all influenza vaccine producing companies is 1.42 billion doses per year, which is not enough to supply the world's population in the case of a pandemic threat [23].

Substitution of the egg-based vaccine production technology by the process based on continuous cell lines offers many advantages including easier scalability and reduced dependence on a large supply of eggs [23]. Cell-based manufacturing for the seasonal influenza vaccine is an alternative production process approved for first time by the FDA in 2012. This process may result in a vaccine containing strains that more closely resemble circulating flu viruses, that match the WHO-selected strains and that can be made more quickly than traditional egg-based vaccines [32, 33]

However, there are some limitations with the cell-based manufacturing process. It requires new production facilities which not all pharmaceutical companies may possess in the case of an influenza pandemic [32, 34]. The vaccine antigenicity could be compromised during the chemical virus inactivation steps [22, 33, 35], and continuing testing must be done to assure the vaccine is free of cell-derived contaminants and other viruses [20, 35]

Also, it has been shown that the cultivation of the human influenza viruses in continuous mammalian cell lines could increase the pH of HA fusion, this dramatically decreases the stability of HA protein, and consequently the whole virion, which can affect the safety, potency, infectivity, and protective efficacy of the final influenza vaccines [23].

All these limitations with the current egg and cell-based vaccines have raised awareness of 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 [29].

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 developed a mosaic nanoparticle vaccine that may provide durable and cross-strain protection against different influenza viruses.

Lumazine synthase is an enzyme that catalyzes the penultimate step of riboflavin synthesis in bacteria, fungi, and plants but not members of the animal kingdom [25, 26]. This enzyme can be used in vaccines for antigen presentation because it mimics the size, shape, multivalency, and symmetric surface geometry of many viruses for improved immunogenicity presentation [27].

Lumazine synthase was engineered as a pentameric scaffold for antigen presentation because it can sterically accommodate 20 HA ectodomain trimers in an orientation that would expose the HA conserved stem which promotes vaccine potency[27] [28].

The FluMos-v1 vaccine is composed of protein-based engineered pentameric scaffold nanoparticles of lumazine synthase from the yeast *Candida albicans (C. albicans*) assembled with 20 HA ectodomain trimers from the following influenza strains: H1, H3, B/Victoria (HBv), and a B/Yamagata (HBy). Unlike previously reported licensed influenza vaccine, this nanoparticle vaccine has been shown to induce both potent receptor-blocking and broadly cross-reactive stem-directed antibody responses in animals, making it an attractive influenza vaccine candidate that might replace the current seasonal vaccines [24].

The advantage of using FluMos-v1 nanoparticles as a vaccine platform is to improve antigen presentation and immune stimulation against different strains of influenza viruses by displaying heterologous antigens on their surface. Furthermore, as the self-assembly process requires no energy and the vaccine 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 [28].

## 1.3. Rationale For Use of the Adjuplex Adjuvant

Adjuvants are vaccine components that enhance the magnitude, breadth, and durability of the immune response. Adjuplex is a novel adjuvant composed of purified lecithin and carbomer homopolymer. Adjuplex is reported to enhance uptake, processing, and cross-presentation of immunogens by dendritic cells [36-39] .

Although Adjuplex is an effective and well-tolerated adjuvant, usable with different antigens and immunization regimens in animals [36, 40], human experience with Adjuplex is limited (see Section 1.6.1).

In mice, soluble influenza hemagglutinin (HA) protein, administered subcutaneously (SC) or intranasally with Adjuplex adjuvant at 1 to20% by volume, induced robust antigen-specific CD8+ T lymphocytes (CTLs) responses [36]. In the mouse Ovalbumin-Adjuplex model,

histological imaging of the IM and SC influenza vaccine injection sites revealed significant positive correlation between dose of Adjuplex and the extent and cellularity of the inflammatory infiltrate at the injection site [36].

Another study in mice corroborated these findings, reporting that soluble influenza A virus HA adjuvanted with Adjuplex and administered SC elicited robust humoral immunity and T-cell responses [40]. The optimal dose of Adjuplex for immunogenicity was 1% by volume; HA adjuvanted with 1% Adjuplex induced high titers of HA-specific IgG with insignificant weight loss and completely protected mice from lethal influenza virus challenge. Adjuplex was comparatively as effective as adjuvants monophosphoryl lipid A (MPL) and alum in preventing disease. No signs of local toxicity or intolerance, assessed by swelling or scratching caused by irritation, were observed at any Adjuplex dose, suggesting the Adjuplex to be a potent and well-tolerated adjuvant for subunit vaccines [40].

Subsequent animal studies have empirically identified 20% Adjuplex as an optimal proportion of adjuvant to immunogen for many formulations. VRC has demonstrated the adjuvant activity of 20% Adjuplex in mice [41, 42], and monkeys [39, 43, 44].

The US Food and Drug Administration recommends that adjuvants be qualified for safety in a Good Laboratory Practices (GLP)-compliant toxicology study. In compliance, VRC has qualified the safety of IM 20% Adjuplex (up to 0.64 ml/dose) administered to rabbits four times at three-week intervals (Q3Wx4) in combination with immunogens (report 294700100101). No treatment-related toxicity was observed, and the results support expectation of safety with use of Adjuplex in VRC 325.

Data from additional nonclinical *in vivo* proof-of-concept and safety studies with 20% Adjuplex in monkeys [45-47] support its use in VRC 325, described below (Section 1.6.1 and Section 2.4).

## 1.4. Previous VRC Influenza Nanoparticle Vaccine Products

The VRC has developed different vaccine platforms that combined bacteria proteins with influenza HA as an antigen display technology [29-31].

Kanekiyo, *et al.* genetically fused the ectodomain of Influenza A/Singapore/1/1957 (H2N2) HA to *H. pylori* ferritin, creating a vaccine that antigenically resembles the native head and stem domains of the H1 HA viral spikes on the surface of the ferritin spherical core. In preclinical immunogenicity studies, this HA ferritin vaccine elicited two types of broadly neutralizing antibodies: against the highly conserved HA stem and antibodies 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 [29].

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 H1 protein on the surface of the ferritin spherical core. 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 [31].

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 [29, 31]

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 [30]. The VRC used these data to design and test a Group 2 HA stem vaccine, described in Section 1.5.3 below.

## 1.5. Previous Human Experience with VRC Influenza Vaccines

### 1.5.1. Human Experience with VRC-FLUMOS0111-00-VP (FluMos-v1)

This is the first Phase I clinical trial (VRC 325; NCT NCT04896086) to evaluate a lumazine synthase particle-based vaccine, FluMos-v1.

Part A opened to accrual on May 24, 2021, and on August 17, 2021, was fully enrolled and closed to accrual. Part B opened to accrual on September 6, 2022, and B final enrollment and product administration occurred on April 20, 2023. No further subjects will be enrolled.

As of March 21, 2023, a total of 42 subjects received the FluMos-v1 vaccine: 31 received FluMos-v1 alone and 11 subjects received FluMos-v1 with Adjuplex. 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.

Solicited Local Reactogenicity

As of March 21, 2023, the most frequent local reactogenicity in the 7 days after FluMos-v1 administration was mild or moderate pain and tenderness at the injection site reported by twenty (20/42, 47.6%) subjects; one (1/5, 20.0%) in the 20 mcg dose Group 1, three (3/15, 20.0%) in the 60 mcg dose Group 2, five (5/11, 45.5%) in the 100 mcg dose Group 4, and 11 (11/11, 100.0%) in the 100 mcg dose plus Adjuplex Group 5, with moderate pain and tenderness reported by one (1/11, 9.1%) Group 5 subject.

Severe redness was reported right after product administration by one (1/11, 9.1%) subject in Group 5 (100 mcg dose plus Adjuplex) that lasted for 1 day. Mild swelling was reported by one (1/11, 9.1%) Group 5 subject. Mild pruritus was reported by two (2/11, 18.2%) Group 5 subjects. No bruising has been reported.

All reported local reactogenicity symptoms resolved within the solicited reactogenicity period of 7 days.

Solicited Systemic Reactogenicity

As of March 21, 2023, systemic reactogenicity symptoms were reported by 19 of 42 (45.2%) subjects. Malaise was the most frequent symptom reported by 17 (17/42, 40.5%) subjects as follows: one (1/5, 20.0%) in the 20 mcg dose Group 1, two (2/15, 13.3%) in the 60 mcg dose Group 2, four (4/11, 36.4%) in the 100 mcg dose Group 4, and ten (10/11, 90.9%) in the 100 mcg dose plus Adjuplex Group 5. Mild malaise was reported by 15 (15/42, 38.1%) subjects: one (1/5 20%) in Group 1, two (2/15, 13.3%) in Group 2, four (4/11, 36.4%) in Group 4, and eight

(8/11, 72.7%) in Group 5. Moderate malaise was reported by two (2/11, 18.2%) Group 5 subjects.

Other reported systemic reactogenicity included mild headache reported by nine (9/42, 21.4%) subjects: two (2/15, 13.3%) in Group 2, two (2/11, 18.2%) in Group 4, five (5/11, 45.5%) in Group 5, and one (1/11, 9.1%) moderate headache in Group 5 that lasted for 12 days; mild chills were reported by ten (10/42, 23.8%) subjects: one (1/5, 20.0%) in Group 1, one (1/15, 6.7%) in Group 2, one (1/11, 9.1%) in Group 4, seven (7/11, 63.6%) in Group 5, and one (1/11, 9.1%) moderate chills in Group 5; mild myalgia reported by eight (8/42, 19%) subjects: one (1/15, 6.7%) in Group 1, one (1/11, 9.1%) in Group 4, six (6/11, 54.5%) in Group 5, and two (2/11, 18.2%) moderate myalgia in Group 5; mild nausea reported by three (3/42, 7.1%) subjects: one (1/15, 6.7%) in Group 2, two (2/11, 18.2%) in Group 5; one of these subjects had mild nausea that started on Day 6 after product administration and lasted for 7 days; mild joint pain reported by four (4/42, 9.5%) subjects: one (1/15, 6.7%) in a Group 2 subject and three (3/11, 27.3%) in Group 5; fever reported by two (2/42, 4.8%) subjects, both in Group 5: one (1/11, 9.1%) mild fever and one (1/11, 9.1%) moderate fever.

The events of mild headache and nausea reported by one 60 mcg dose Group 2 recipient resolved 8 days after product administration. The one event of mild joint pain reported by a subject in the 60 mcg dose Group 2 resolved 97 days after product administration and the persistent symptoms beyond the solicited reactogenicity period were consistent with an overuse injury with no signs or symptoms of inflammation, including lack of swelling, erythema, fever, or any systemic symptoms. The participant had no other unsolicited adverse events and negative inflammatory markers including normal WBC, ESR, CRP, ANA, RF, and anti-CCP, were within normal limits.

One Group 5 subject experienced mild headache, chills, and nausea that lasted for a total of 7-12 days. One Group 5 subject experienced mild headache on day 1 that increased to moderate on Day 2 and was associated with moderate malaise, chills, myalgia, and mild pain and tenderness that all resolved on Day 4 with mild headache recurring on Day 6 that resolved the same day. There was no associated joint pain or fever.

All other systemic reactogenicity symptoms resolved within the 7-day solicited period.

Unsolicited Adverse Events

Among the 42 FluMos-v1 vaccine recipients, 19 of 42 (45.2%) subjects experienced at least one unsolicited AE, with most events reported as mild in severity. The maximum severity for unsolicited AEs that was experienced by participants with one or more AEs was mild for 14 of 42 (33.3%) subjects, moderate for 3 of 42 (7.9%) subjects, severe for 1 of 42 (2.4%) subjects and life-threatening for 1 of 42 (2.4%) subjects. There were no AEs that met the criteria for expedited reporting.

Mild to severe anemia was the most frequently reported AE, reported for 6 of 42 (14.3%) FluMos-v1 vaccine recipients and assessed as not related to study product. Six anemias (3 mild, 2 moderate, and 1 severe) were evaluated as related to blood loss from study-related blood collection and were not related to the study product.

One anemia for a subject in the 20 mcg FluMos-v1 Group 1 was mild by absolute hemoglobin value but was graded as severe due to the decrease to 13.1 gm/dL from a baseline of 15.3 gm/dL; this anemia resolved in 2 weeks without sequalae. One mild anemia occurred in another 20 mcg

FluMos-v1 Group 1 subject with a baseline hemoglobin on the lower end of the institutional normal range, and it resolved without sequelae 10 weeks after study product administration, after the subject had no blood drawn for 8 weeks. One moderate anemia in a 60 mcg FluMos-v1 Group 2 subject with a baseline hemoglobin on the lower end of the institutional normal range was detected 28 days after study vaccination; this event was graded as moderate due to a 1.6 gm/dL decrease from baseline, and attribution was related to study-related blood collection. Three anemias occurred in three 60 mcg FluMos-v1 Group 2 participants: one mild detected 12 days after product administration and two moderate detected 12 and 28 days after product administration. The anemias resolved in 16, 99 and 56 days, respectively. One mild anemia in a 100 mcg dose FluMos-v1 plus Adjuplex Group 5 participant was detected 14 days after product administration, resolved in 70 days, and attribution was most likely related to the subject's vegetarian diet and blood loss from study blood collection, and not related to study product.

One case of mild influenza-like illness (ILI) was reported for the Group 5 subject (100 mcg FluMos-v1 plus Adjuplex) that was confirmed to be an infection with a coronavirus NL63 by a respiratory panel testing. One life-threatening SAE of a brain tumor was reported for a subject in the 60 mcg FluMos-v1 Group 2, 121 days after product administration, and was assessed as unrelated to study product. No other SAEs were reported. There were no AEs that met the criteria for expedited reporting and no events that led to a study pause.

Five (5/42, 11.9%) FluMos-v1 vaccine recipients experienced unsolicited AEs that were evaluated as related to study product. Two subjects had mild lymphadenopathy, two subjects had transient mild to moderate neutropenia, and one subject had mild lymphopenia as follows:

- One subject in the 60 mcg FluMos-v1 Group 2 had mild cervical lymphadenopathy ipsilateral to the site of injection 3 days after product administration, which resolved without sequelae. Several small non-tender lymph nodes were initially palpable, and all resolved within 2 weeks except one, which was initially estimated at 0.5 cm and gradually decreased in size until complete resolution 83 days after onset.
- One subject in the 60 mcg FluMos-v1 Group 2 had mild cervical lymphadenopathy 12 days after the product administration, which resolved without sequelae the same day.
- Two subjects in the 20 mcg FluMos-v1 Group 1 had mild neutropenia 14 days after product administration, which resolved without sequelae 14 days after onset.
- One subject in the 100 mcg dose FluMos-v1 without Adjuplex Group 4 had mild lymphopenia 27 days after product administration, which resolved 33 days after onset.

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.

Also, human experience with the ferritin nanoparticle-based vaccine platforms: VRC-FLUNPF081-00-VP (HA-F A/Sing), VRC-FLUNPF099-00-VP (H1ssF_3928), and VRC-FLUNPF0103-00-VP (H10ssF-6473) is provided below.

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

The first Phase I clinical trial (VRC 316; NCT03186781) to evaluate a ferritin particle-based vaccine, 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. Group 1 received a single 20-mcg dose at Day 0. Group 2 received a 60-mcg dose at Day 0 and Week 16. The study data have been recently published [48].

### 1.5.3. Previous Human Experience with VRC-FLUNPF099-00-VP (H1ssF_3928)

The second Phase I clinical trial (VRC 321; NCT03814720) for evaluation of ferritin nanoparticle vaccine, the H1ssF_3928 ferritin vaccine, opened to accrual in April 2019. 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 the H1ssF_3928 vaccine included 2 dose groups. Group 1 received a single 20-mcg dose at Day 0. Group 2 received a 60-mcg dose of product at Day 0 and Week 16. The study data have been recently published [49].

### 1.5.4. Previous Human Experience with VRC-FLUNPF0103-00-VP (H10ssF-6473)

The third Phase I clinical trial (VRC 323; NCT04579250) for evaluation of stabilized stem ferritin nanoparticle vaccine, the H10ssF-6473 vaccine, opened to accrual on October 8, 2020. H10ssF-6473 vaccine is composed of *H. pylori* non-heme ferritin genetically fused to the influenza virus H10 HA to form a nanoparticle that antigenically resembles the native stabilized stem domain of the HA from A/Jiangxi/IPB13/2013 (H10N8) influenza strain. Evaluation of the H10ssF-6473 vaccine included 2 dose groups. Group 1 received a single 20-mcg dose at Day 0. Group 2 received a 60-mcg dose at Day 0 and Week 16. The study data have been recently published in ClinicalTrials.gov at https://clinicaltrials.gov/results/NCT04579250.

As of September 14, 2022, the study was completed, and the database is locked. All 25 healthy adults 18-70 years of age received the injection of the H10ssF-6473 vaccine. The first study enrollment and product administration occurred on October 8, 2020, the final enrollment occurred on April 22, 2021, and the final study visit occurred in January 2022. All injections with the H10ssF-6473 vaccine were well tolerated.

Maximum local reactogenicity in the 7 days after H10ssF-6473 administration was mild and there were no reports of fever. No solicited local reactogenicity symptoms were reported for subjects enrolled in Group 1 who received 20 mcg of H10ssF-6473 (n=3). The most frequently reported solicited local reactogenicity in this study was mild pain and tenderness at the injection site. In Group 2A (60 mcg of H10ssF-6473, two doses, age cohort 18-50) 3 of 14 (21.4%) participants report mild pain and tenderness at the injection site after the first injection, and 3 of 14 (21.4%) reported mild pain and tenderness at the injection site after the second vaccination, In Group 2B (60 mcg of H10ssF-6473, two doses, age cohort 55-70) 1 of 8 (12.5%) individuals reported mild pain and tenderness at the injection site after the first vaccination, and 2 of 8 (25%) reported mild pain and tenderness at the injection site after the second vaccination. None of the participants in Group 2A reported swelling at the injection site after either vaccination, and 1 of 8 (12.5%) participants in Group 2B reported mild swelling at the injection site after the first vaccination. No symptoms of redness, pruritis or bruising were reported by any trial participants. All local reactogenicity symptoms resolved within the solicited period.

Regarding solicited systemic reactogenicity, mild malaise was the most frequently reported systemic reactogenicity in this study with 10 of 25 (40.0%) subjects reporting symptoms of mild malaise. In Group 1 (20 mcg of H10ssF-6473, single dose), 1 of 3 subjects (33%) reported mild malaise post-vaccination. In Group 2A (60 mcg of H10ssF-6473, repeat dose), 3 of 14 subjects (21.4%) reported mild malaise after the first vaccination and 4 of 14 (28.6%) reported mild malaise after the second vaccination. None of the 8 subjects in Group 2B (60mcg of H10ssF-6473, repeat dose), reported any symptoms of malaise after either the first or second dose. None of the 3 participants in Group 1 reported post-vaccination myalgias. In Group 2A, 3 of the 14 participants (21.4%) reported mild myalgias after the first vaccination, and 1 of 14 (7.1%) reported mild myalgias after the second vaccination. In Group 2B, only 1 of 8 (12.5%) participants reported mild myalgia after the first vaccination, and none were reported after the second vaccination. In Group 2A, a mild headache was reported by 1 of 14 participants (7.1%) but only after the second vaccination. In Group 2B, 1 of 8 (12.5%) participants reported mild headaches after the first vaccination, and 1 of 8 (12.5%) participants also reported a mild headache after the second vaccination. In Group 2A, mild chills were reported after the first and second vaccination by 1 of 14 participants (7.1%), and none were reported in Group 2B. In Group 2B mild nausea was reported by 1 of 8 participants (12.5%) after the second vaccination. In Group 2A mild joint pain was reported by 1 of 14 participants (7.1%) after the first vaccination. Systemic reactogenicity symptoms resolved within the solicited period for all participants except for one individual in Group 2A. In this individual, mild malaise after the second vaccination was reported on Day 6 and resolved 2 days later on Day 8 (one day after the solicited period).

Overall, 18 of 25 (72%) subjects had one or more unsolicited AE with maximum severity being mild for 11 of 25 (44%) subjects, moderate for 6 of 25 (24%) subjects, and severe for 1 of 25 (4%). The most frequently occurring event was anemia in 4 of 25 (16%) subjects.

There were five events that were determined to be related to study product. One Group 1 (20 mcg of H10ssF-6473) subject had moderate neutropenia 28 days after product administration that could not be followed up to resolution since this subject was lost to follow up. Four subjects in Group 2A (60 mcg of H10ssF-6473, repeat dose) had mild AEs as follows: 1 subject had neutropenia (33 days after the first product administration which resolved 51 days after onset), 1 subject had lymphopenia (28 days after the first product administration which resolved 56 days after onset), 1 subject had aspartate aminotransferase (AST) increase (28 days after the second product administration which resolved 14 days after onset), and 1 subject had leukopenia (28 days after the first product administration which resolved 56 days after onset). These 4 AEs were followed to resolution, and all resolved without sequelae.

### 1.5.5. Human Experience with QIV Flucelvax®

More information regarding QIV Flucelvax® can be found in the US Package Insert [50].

The safety of Flucelvax® was evaluated in seven randomized, controlled studies conducted in the US, Europe, and New Zealand. In all studies, solicited local injection site and systemic adverse reactions were collected from subjects who completed a symptom diary card for 7 days following vaccination.

Per the Flucelvax® Revision 7 of the package insert as of March 2020, the most common local solicited adverse reactions occurring in adults 18 to 64 years of age within 7 days after

vaccination with Flucelvax® were pain (45.4%), erythema (13.4%), ecchymosis (3.8%) and induration (11.6%) at the injection site. Systemic AEs were: chills (6.2%), nausea (9.7%), myalgia (15.4%), arthralgia (8.1%), headache (18.7%), fatigue (17.8%), vomiting (2.6%), diarrhea (7.4 %), loss of appetite (8.3%), and fever (0.8%) [50].

Flucelvax® efficacy was assessed by the prevention of culture-confirmed symptomatic influenza illness caused by viruses antigenically matched to those in the vaccine and prevention of influenza illness caused by all influenza viruses compared to placebo. Influenza cases were identified by active and passive surveillance of influenza-like illness (ILI). ILI was defined as a fever (oral temperature ≥100.0°F / 38°C) and cough or sore throat. Nose and throat swab samples were collected for analysis within 120 hours of onset of an ILI in the period from 21 days to 6 months after vaccination. Overall vaccine efficacy against all influenza viral subtypes and vaccine efficacy against individual influenza viral subtypes were 83.8% for antigenically matched strains and 69.5% for all culture-confirmed influenza [50].

Immunogenicity data in adults 18 through 64 years of age were derived from Phase II and III clinical studies (NCT 0063033, NCT 00492063, and NCT 00264576) that included 1353 subjects who received Flucelvax®. Immune responses measured by hemagglutination inhibition (HI) antibody titers to each virus strain in the vaccine were evaluated in sera obtained 21 days after administration of Flucelvax® or egg-based comparator vaccine. For all outlined studies, antibody responses after vaccination were evaluated according to percentages of subjects with HI antibody titers and seroconversion.

For NCT 0063033 and NCT 00492063 phase III studies, Flucelvax® (previously known as Optaflu) and Agippal (egg-derived vaccine) were highly immunogenic, with post-vaccination responses to influenza type B slightly higher in the Agippal group [51], the statistical comparison between these two vaccines demonstrated that Flucelvax® was noninferior to the conventional egg-based control vaccine [52]. For NCT 00264576 Phase II study, Flucelvax® and Fluvirin (egg-derived vaccine) showed that the post-vaccination responses to influenza A/H1N1 strain were equivalent for both vaccines, higher to A/H3N2 strain in the Fluvirin group and to influenza type B in the Flucelvax® group [53].

In conclusion, sero-protection rates, seroconversion rates, and geometric mean ratios after vaccination with Flucelvax® and all the vaccine virus stains exceeded both European and the USA FDA immunogenicity licensing criteria.

### 1.5.6. Flucelvax® Post-marketing Experience

The following additional adverse events have been identified during post-approval use of Flucelvax®: Immune system disorders (anaphylactic reaction, angioedema), skin and subcutaneous tissue disorders (generalized skin reactions including pruritus, urticaria or non-specific rash), nervous systems disorders (syncope, presyncope, paresthesia), and general disorders and administration site conditions (extensive swelling of the injected limbs).

These events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine [50].

## 1.6. Rationale for Study Products, Dose, and Influenza Strains Selection

### 1.6.1. Previous Human Experience with Adjuplex

Adjuplex has been tested in the following studies:

- This Phase 1 clinical study of FluMos-v1,
- An ongoing Phase 1 clinical study of an adenovirus-vectored vaccine against cocaine, NCT02455479, and
- A suspended Phase 1 clinical trial of HIV envelope fusion peptide and trimer vaccines (HVTN 303, NCT05470400).

A summary of the cocaine vaccine study (NCT02455479) objectives and endpoints, as well as study contact information, can be found on the ClinicalTrials.gov website.

The HVTN 303 study evaluated three candidate HIV-1 vaccines formulated with Adjuplex (20% v/v). This study was paused on January 11, 2023, and subsequently placed on clinical hold by the FDA on March 13, 2023, due to safety trends of increased severity and frequency of reactogenicity and unsolicited adverse events that were expected, including rash, urticaria, transient elevation of the absolute eosinophil count, and one event of serum sickness. There have been no SAEs or SUSARs reported in the study. The protocol team concluded that the constellation of observed events appeared to be specific to the antigen and adjuvant combinations used in the HVTN 303 study. An investigation is ongoing to further investigate the potential causes of the adverse reactions that have occurred in this study.

Safety data collected so far in the VRC 325 study are described in Section 1.5.1. To date, besides the transient episode of severe redness that occurred at the injection site of a subject in Group 5, the solicited reactogenicity related to FluMos-v1 plus Adjuplex was mild to moderate in severity. All local reactogenicity events for Group 5 resolved within the 7-day solicited period. The majority of systemic reactogenicity events resolved within the 7-day period, except for one subject who experienced mild headache, chills, and nausea that lasted for a total of 6-12 days after product administration. There have been no events that led to a study pause and no SAEs or SUSARs have occurred in this study.

All study enrollments and injections have been completed for groups 1-5 on this study as of April 20, 203, and a decision was made not to enroll the optional VRC 325 Groups 6-8.

### 1.6.2. FluMos-v1 Vaccine

VRC 316, VRC 321 and VRC 323 studies showed that two dose levels of the ferritin nanoparticle-based vaccines, 20 mcg and 60 mcg of HA-F A/Sing, of H1ssF_3928 and of H10ssF-6473 respectively, were safe and well tolerated. The same vaccine doses were also evaluated in preclinical evaluations of FluMos-v1 vaccine as discussed in Section 2.4.

In pre-clinical studies, addition of adjuvant increased the immunogenicity of FluMos-v1.

In study VRC 325 Part A, FluMos-v1 vaccine was evaluated at 20 mcg and 60 mcg as a single dose. The dose levels chosen to be tested clinically were selected based on the preclinical immunogenicity studies. Both the 20 mcg and 60 mcg doses were safe and well tolerated as described in Section 1.5.1. Immunogenicity analysis of Part A is still ongoing, but preliminary

data indicate the need to increase the dose and/or add an adjuvant to improve immunogenicity. Given the safety profile of 60 mcg, and toxicology studies supporting doses of up to 190 mcg, in Part B, the VRC will test a single dose of 100 mcg with and without adjuvant, with optional Part C to test a single dose up to 190 mcg, after IRB approval. An initial dose of 100 mcg was chosen for Part B because it has an HA protein content of approximately 62 mcg, which more closely matches the HA content of Flucelvax (60 mcg of HA).

Since the nanoparticle itself makes up close to 40% of the total weight, our initial dose of 60 mcg was not equivalent to the QIV dose per the HA quantity. Therefore, the original 60 mcg dose is not an ideal comparison to the licensed QIV. The 100 mcg dose (Group 4) was included to evaluate the safety of a higher dose of FluMosv-1 on its own before the addition of adjuvant. If we observe adverse safety signals with the 100 mcg dose alone or with Adjuplex, we will stop administering this dose as described in the protocol Section 4.7, and may enroll the 60 mcg plus Adjuplex group, which is built into the protocol as an optional group for additional safety and immunogenicity assessments.

Influenza H1, H3, HBv, and Hby HA strains were selected for evaluation based on the influenza lineage types included in the licensed 2019-2020 seasonal QIV vaccine.

### 1.6.3. Flucelvax® Vaccine

Flucelvax® vaccine was selected to evaluate the immunogenicity and dosing regimen of the FluMos-v1 vaccine because Flucelvax® is the licensed 2020-2021 seasonal QIV. Flucelvax® is a cell-based vaccine which contains the same four influenza strains selected by the WHO and has shown better safety and immunogenicity profiles that the traditional licensed seasonal egg-based vaccine [48].

### 1.6.4. Adjuplex

HIV vaccine candidates adjuvanted with the Adjuplex dose of 20% by volume were found to elicit reproducible, neutralizing immune responses against diverse tier-2 primary isolates in a prime-boost regimen in animals [54]. Boosting with Trimer 4571 after Fusion Peptide priming, with Adjuplex adjuvant, was found to significantly enhance neutralizing titers, especially in guinea pigs and rhesus macaques, with vaccine-elicited rhesus antibodies neutralizing up to 59% of the 208-strain HIV pseudoviral panel [39].

VRC evaluated the safety and immunogenicity of FluMos-v1 with 20% v/v Adjuplex (same formulation and volume ratio to be used clinically) in a non-GLP study in mice and rhesus macaques vaccinated IM on weeks 0 and 4, with blood collection pre-dose, and post-dose at weeks 2 and 6. The vaccine was well tolerated with and without Adjuplex, with no mortality, abnormal clinical signs, or changes in body weight. Body temperature was measured in monkeys and was not affected by treatment. In mice, the addition of Adjuplex to the vaccine resulted in increased HA-binding antibody response, improved antibody responses against the influenza groups 1 and 2 HA subtype stems, and increased virus neutralizing antibody response to vaccine-matched viruses. No effect of treatment was observed. In monkeys, a single dose of FluMos-v1 with Adjuplex elicited robust immune responses, and the responses were increased after the second dose of the combination. After the second dose, the combination of vaccine with Adjuplex induced statistically significant increases in HA-specific IgG, microneutralization, and HAI in monkeys compared to the vaccine alone.

Adjuplex was chosen as the adjuvant for this trial because it performed favorably with FluMos-v1 when compared with other adjuvants in preclinical studies.

## 1.7. Rationale For Study Population

Healthy subjects 18-50 years of age inclusive will be enrolled to assess safety, tolerability, and immunogenicity of the FluMos-v1 vaccine. This subject population was chosen to evaluate the immune responses between Flucelvax® and FluMos-v1 because studies has shown that adults 55 years and older have lower antibody responses when vaccinated with Flucelvax® [50].

## 1.8. 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 point is two weeks after product administration. The immunogenicity will be assessed by detection of HA responses to influenza H1, H3, HBv, and Hby HA strains using the Meso-Scale Discovery (MSD) or similar platform.

Additional assessment of HA specific antibody responses may be conducted on stored samples obtained throughout the study.

Exploratory evaluations may include measurements of antibody response to the engineered pentameric scaffold of lumazine synthase from *C. albicans* and engineered trimer at various time points throughout the study.

Additional exploratory evaluations may include the detection of neutralizing antibodies to influenza by neutralization and functional serological assays, and exploratory B and T cell assays.

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

Some immunogenicity assays may be performed by the 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 to show proof-of-concept for elicitation of antibody responses by a quadrivalent mosaic vaccine and contribution of a novel adjuvant, Adjuplex, to vaccine immunogenicity.

# 2. STUDY PRODUCTS

## 2.1. VRC-FLUMOS0111-00-VP

The VRC-FLUMOS0111-00-VP (FluMos-v1) vaccine is composed of engineered pentamer yeast *C. albicans* lumazine synthase assembled with 20 HA ectodomain trimers from the following influenza strains:

**Influenza A**: H1: A/Idaho/07/2018

H3: A/Perth/1008/2019

**Influenza B**: B/Victoria lineage: B/Colorado/06/2017

B/Yamagata lineage: B/Phuket/3073/2013

VRC-FLUMOS0111-00-VP (FluMos-v1) is manufactured under current Good Manufacturing Practice (cGMP) at the VRC Pilot Plant by Leidos Biomedical Research, Inc., Frederick, MD. FluMos-v1 vaccine is a sterile, aqueous buffered solution. Vials are aseptically filled to a volume of 0.7 mL into a single dose vial at 180 mcg/mL.

More details related to vaccine production, quality control and preclinical studies performed with FluMos-v1 vaccine can be found in the Investigator's Brochure (IB).

## 2.2. FLUCELVAX® QIV

Flucelvax® is the 2020-2021 seasonal influenza vaccine licensed by the FDA indicated in persons 4 years of age and older. The vaccine is composed of the following influenza strains:

**Influenza A**: H1: A/Hawaii/70/2019 (H1N1)pdm09-like virus

H3: A/Hong Kong/45/2019 (H3N2)-like virus

**Influenza B**: B/Victoria lineage: B/Washington/02/2019-like virus

B/Yamagata lineage: B/Phuket/3073/2013-like virus

Flucelvax® is manufactured under cGMP by Seqirus Inc. This vaccine is produced in Madin Darby Canine Kidney (MDCK) cells and inactivated using ß-propiolactone. Each of the four virus strains is produced and purified separately then pooled to formulate the QIV. Each dose of Flucelvax® may contain residual amounts of MDCK cell protein, protein other than HA, MDCK cell DNA and β-propiolactone. Flucelvax® provided in single doses contains no preservative or antibiotics.

## 2.3. Adjuplex, VRC-GENADJ0110-AP-NV

Adjuplex is a sterile, pyrogen-free adjuvant solution produced by VRC Pilot Plant. Adjuplex comprises highly purified de-oiled soy lecithin and benzene-free carbomer homopolymer formulated in phosphate buffered saline at a pH of 6.5 ± 0.3 (6.2-6.8). Adjuplex adjuvant will be mixed with FluMos-v1 at 20% by volume in the pharmacy during product preparation for the vaccinations of Groups 5A-5B. The decision was made not to enroll groups 6A-6B, and 8A-8B.

## 2.4. Preclinical Studies with FluMos-v1 Vaccine

Preliminary non-clinical immunogenicity studies in mice, ferrets, and nonhuman primates demonstrated that FluMos vaccine administered IM elicited more potent hemagglutination inhibition and virus neutralizing antibody responses to vaccine-matched strains than did commercial QIV. Also, the immune sera elicited by the mosaic nanoparticle vaccine neutralized a wide spectrum of Influenza A viruses across both Group 1 and Group 2, including H5N1 and H7N9 subtypes, and a substantial portion of this heterosubtypic neutralization was mediated by mAbs targeting the conserved HA stem supersite. When passively administered prophylactically to mice, immunoglobulins purified from macaques immunized with the mosaic nanoparticle also provided protection against lethal heterosubtypic H5N1 and H7N9 challenge [24].

The safety of FluMos-v1 was evaluated in a toxicology study using New Zealand White Rabbits administered IM twice at three week intervals for two injections. In summary, IM vaccination with FluMos-v1 at doses of 64.7 and 194 mcg for two injections on study Days 1 and 22 resulted in no treatment-related, toxicologically significant, or adverse findings. More details on this study and additional pre-clinical studies with FluMos-v1 can be found in the IB.

For adjuvanted groups in Part B and C, the immunogenicity of Adjuplex-adjuvanted FluMos-v1was evaluated in non-clinical studies in mice and non-human primates (NHPs). FluMos-v1 alone or FluMos-v1 plus Adjuplex were well tolerated, with no discernible effects on body weight, morbidity, mortality, or clinical signs. Measured body temperature in NHP was unaffected by FluMos-v1 vaccination, with or without Adjuplex. In mice, the measured responses elicited by FluMos-v1 were consistent with those in the previous non-clinical study.

The addition of Adjuplex to the vaccine resulted in increased HA-binding antibody response, improved antibody responses against the influenza groups 1 and 2 HA subtype stems, and increased virus neutralizing antibody response to vaccine-matched viruses. In NHPs, two doses of FluMos-v1 alone elicited immune responses by all three assays, HA-specific IgG, microneutralization, and HAI. A single dose of FluMos-v1 plus Adjuplex elicited robust immune responses, and the responses were increased after the second dose of the combination. There was also a significant increase in virus neutralizing activity in sera from the adjuvanted group in all four vaccine matched strains of virus at both time points. More details on these studies and additional pre-clinical studies with FluMos-v1 can be found in the IB.

# 3. STUDY OBJECTIVES

## 3.1. Primary Objectives

**Part A:**

- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered as a single dose at 20 mcg IM via needle and syringe to healthy adults.
- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered as a single dose at 60 mcg IM via needle and syringe to healthy adults.

**Part B:**

- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered alone as a single dose at 100 mcg IM via needle and syringe to healthy adults.
- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered as a single dose at 100 mcg with Adjuplex at 20% v/v IM via needle and syringe to healthy adults.
- If enrolled, to evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered as a single dose at 60 mcg with Adjuplex at 20% v/v IM via needle and syringe to healthy adults.

**Part C (if enrolled):**

- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered alone as a single dose at TBD mcg IM via needle and syringe to healthy adults.
- To evaluate the safety and tolerability of the VRC-FLUMOS0111-00-VP vaccine, administered as a single dose at TBD mcg with Adjuplex at 20% v/v IM via needle and syringe to healthy adults.

## 3.2. Secondary Objectives

**Part A:**

- To evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered as a single dose at 20 or 60 mcg IM via needle and syringe at two weeks after injection.
- To evaluate antibody responses between VRC-FLUMOS0111-00-VP administered as a single dose at 60 mcg IM via needle and syringe and Flucelvax® licensed vaccine at two weeks after vaccination.

**Part B:**

- To evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered alone as a single dose at 100 mcg IM via needle and syringe at two weeks after injection.

- To evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered as a single dose at 100 mcg with 20% v/v of Adjuplex IM via needle and syringe at two weeks after injection.
- To evaluate antibody responses between 100 mcg of VRC-FLUMOS0111-00-VP alone and 100 mcg of VRC-FLUMOS0111-00-VP with 20%v/v of Adjuplex IM via needle and syringe at two weeks after vaccination.
- If Group 6A-6B is enrolled, evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered as a single dose at 60 mcg with 20% v/v of Adjuplex IM via needle and syringe at two weeks after injection.

**Part C (if enrolled):**

- To evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered alone as a single dose at TBD mcg IM via needle and syringe at two weeks after injection.
- To evaluate antibody responses to the VRC-FLUMOS0111-00-VP vaccine administered as a single dose at TBD mcg with 20% v/v of Adjuplex IM via needle and syringe at two weeks after injection.
- To evaluate antibody responses between TBD mcg of VRC-FLUMOS0111-00-VP alone and TBD mcg of VRC-FLUMOS0111-00-VP with 20%v/v of Adjuplex IM via needle and syringe at two weeks after vaccination.

## 3.3. Exploratory Objectives

- To evaluate the specificity and functionality of VRC-FLUMOS0111-00-VP and Flucelvax® vaccine-induced antibodies and the immune response at various timepoints throughout the study.
- To evaluate the frequency, magnitude, phenotype, and specificity of HA-specific B-cell, T-cell, and antibody responses at various time points throughout the study.
- To evaluate the antibody response to the pentameric scaffold lumazine synthase and engineered trimer at various time points throughout the study.

# 4. STUDY DESIGN, SUBJECT POPULATION AND CLINICAL PROCEDURES

## 4.1. Study Design

This is a Phase I, open-label, dose escalation study to evaluate the dose, safety, tolerability, and immunogenicity of the FluMos-v1 vaccine with or without Adjuplex in healthy adults. The study schema is shown in **Table 1**. The hypotheses are that the vaccine with or without Adjuplex is safe, tolerable, and will induce an antibody response to the four influenza strains: H1, H3, HBv, and Hby. The study will be conducted by the VRC Clinical Trials Program at a single site (Vaccine Evaluation Clinic, VEC) at the NIH Clinical Center (NIH CC), Bethesda, MD.

**Table 1: Study Schema**

| VRC 325 Vaccination Schema | | | |
|---|---|---|---|
| **Group** | **Subjects** | **Day 0** | **Product** |
| **Part A** | | | |
| 1A<br>1B[1] | **5** | 20 mcg | FluMos-v1 |
| 2A<br>2B[1] | **15** | 60 mcg | FluMos-v1 |
| 3A<br>3B[1] | **15** | 60 mcg | Flucelvax® |
| **Part B** | | | |
| 4A<br>4B[2] | **13** | 100 mcg | FluMos-v1 |
| 5A<br>5B[2] | **13** | 100 mcg | FluMos-v1 + Adjuplex (20% v/v) |
| 6A<br>6B[2] | **13** | [3]60 mcg | FluMos-v1 + Adjuplex (20% v/v) |
| **[3]Part C** | | | |
| 7A<br>7B[2] | **13** | [3]X mcg | FluMos-v1 |
| 8A<br>8B[2] | **13** | [3]X mcg | FluMos-v1 + Adjuplex (20% v/v) |
| **Total** | **100** [4] | [1]Includes subjects who received the 2020-2021 seasonal influenza vaccine.<br>[2]Includes subjects who received the 2021-2022 or 2022-2023 seasonal influenza vaccine.<br>[3]Group 6 and Part C of the study are optional, and the decision was made not to enroll groups 6-8.<br>[4]Study enrollment has been completed with 35 subjects enrolled in Part A (Groups 1-3) and 26 in Part B (Groups 4-5) of the study. | |

In Part A and Part B, the study had staged enrollment with required interim safety reviews, as described in Section 4.5, Criteria for Dose Escalation.

Subjects enrolled in Group 3A-3B enrolled at any time after the study was open to accrual as they received only a licensed influenza vaccine.

The optional Group 6 and Part C of the study (Groups 7-8) were not enrolled in the study.

## 4.2. Study Population

All inclusion and exclusion criteria must be evaluated for eligibility.

### 4.2.1. Inclusion Criteria

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

1. Healthy adults between the ages of 18-50 years inclusive
2. Based on history and physical examination, in good general health and without history of any of the conditions listed in the exclusion criteria
3. **Part A:** Received at least one licensed influenza vaccine from 2016 through the 2019-2020 influenza season
4. **Part B** and **C**: Received at least one licensed influenza vaccine from 2017 through the 2022-2023 influenza season
5. Able and willing to complete the informed consent process
6. Available for clinic visits for 40 weeks after enrollment and through an influenza season
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) ≤ 35 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/μL
11. Platelets = 125,000 – 500,000 cells/μL
12. Hemoglobin within institutional normal range or accompanied by the PI or designee approval
13. Alanine aminotransferase (ALT) ≤ 1.25 x institutional upper limit of normal (ULN)
14. Aspartate aminotransferase (AST) ≤ 1.25 x institutional ULN
15. Alkaline phosphatase (ALP) <1.1 x institutional ULN
16. Total bilirubin within institutional normal range or accompanied by the PI or designee approval.
17. Serum creatinine ≤ 1.1 x institutional ULN
18. Negative for HIV infection by an FDA-approved method of detection

***Criteria applicable to women of childbearing potential***:

19. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) on the day of enrollment

20. Agrees to use an effective means of birth control from at least 21 days prior to enrollment through the end of the study

### 4.2.2. 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 H1, H2, or H10 influenza vaccines, including Part A participants
10. **Part A**:
    a. Groups 1A, 2A, and 3A only: Receipt of the 2020-2021 season's licensed influenza vaccine at any time prior to enrollment
    b. Groups 1B, 2B, and 3B only: Receipt of the 2020-2021 season's licensed influenza vaccine within 4 months prior to enrollment.
11. **Part B** and **C**:
    a. Groups 4A, 5A, 6A, 7A, and 8A only: Receipt of the 2021-2022 or 2022-2023 season's licensed influenza vaccine at any time prior to enrollment
    b. Groups 4B, 5B, 6B, 7B, and 8B only: Receipt of the 2021-2022 or 2022-2023 season's licensed influenza vaccine within 4 months prior to enrollment.

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

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

19. Hypertension that is not well controlled
20. 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
21. Malignancy that is active or history of malignancy that is likely to recur during the period of the study
22. 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
23. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen
24. Guillain-Barré Syndrome
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.

## 4.3. Inclusion of Vulnerable Subjects

### 4.3.1. 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.

### 4.3.2. Adult Subjects who Lack Capacity to Consent to Research Participation

Adults who are unable to provide initial informed consent are excluded to enroll. Also, adults who permanently lose the capacity to provide on-going consent after initial consent and during the study will be discontinued from protocol participation as it is described in Section 4.6.

### 4.3.3. 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 FAQs for NIH Staff Who are Considering Participation in NIH Research" published by Office of Human Research Subjects Protections on Research Involving NIH Staff as Subjects, Policy 404.

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. Study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

## 4.4. 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 injection.

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.

### 4.4.1. Recruitment and Retention Strategies

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

### 4.4.2. Costs

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

### 4.4.3. 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 the visit that includes the administration of the study product and $200 for clinic visits that include a blood draw (with or without oral mucosal sample collection). Any visit that includes specimen collection with nasopharyngeal swabs will result in an additional $55 in compensation. The compensation for any clinic visit that does not include nasopharyngeal sample or any specimen 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 completed, performance of optional research blood collections and timely submission of electronic diary cards.

The approximate total compensation for all Groups is $1,940 without apheresis and $2,025 if apheresis is performed. Subjects will usually 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.

### 4.4.4. Screening

All screening procedures 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, HIV, 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 under screening protocol, VRC 500. Additional assessments of health may be conducted during screening based on clinical judgment. Screening evaluations for specific eligibility criteria (Section 4.2) must be completed within the time interval specified prior to enrollment for the given parameter and may be repeated, as needed, to confirm eligibility. No screening procedures will be done under protocol VRC 325.

Research blood samples may be collected at any time 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 325. Records will be kept documenting the reason if screened subjects do not enroll.

### 4.4.5. 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 study injection. The clinicians will discuss the target dates and timing of 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.

### 4.4.6. Enrollment and Study Day 0

Day 0 is defined as the day of protocol enrollment and 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 that significant changes 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 injection are the baseline for subsequent safety assessments.

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. All subjects that receive FluMos-v1, Flucelvax®, or

FluMos-v1 with Adjuplex will be expected to continue with follow-up through the end of the study.

### 4.4.7. Pregnancy: Acceptable and Effective Methods of Birth Control

Women of reproductive potential must commit to use an effective method of birth control, beginning 21 days prior to enrollment and continuing 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.

If a pregnancy occurs during the course of the study, it will be recorded in the study database. Any pregnant participant will have no further research sample collections or procedures but will continue to be followed for clinical safety and to collect the pregnancy outcome. Any follow-up procedures and/or data collected will be for clinical/safety outcome purposes only. Pregnancy will be reported as described in Section 5.4.

### 4.4.8. 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 vaccination.

On the day of and prior to product administration, 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 or injury that precludes administration of the vaccine may be rescheduled within the allowed study visit window.

A negative pregnancy test result for women of reproductive potential must be obtained on the day of enrollment and prior to vaccination.

When choosing an arm for injection, clinicians will assess for injury, local skin conditions or tattoos that preclude administration or may interfere with evaluation of the injection site.

### 4.4.9. Post-Product Administration Follow-Up

All subjects will be observed for a minimum of 30 minutes following vaccination. Vital signs (temperature, blood pressure, pulse, and respiratory rate) and assessment of local reactogenicity will be performed after product administration.

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.

### 4.4.10. 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 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 reactogenicity signs and symptoms on the diary card will include the following systemic symptoms: generalized symptoms of unusually tired/feeling unwell, fever, muscles aches (other than at injection site), headache, chills, nausea, and joint pain. The following local reactogenicity symptoms will be included: pain/tenderness, redness, swelling, pruritus and bruising at the injection site.

Each day, subjects will also record their highest measured temperature and measurement of the largest diameter of redness, swelling, and/or bruising at the injection site.

Follow-up on subject well-being will be performed by telephone on the first or second day following vaccination 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 product administration 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.

### 4.4.11. Follow-Up through End of Study

Study follow-up will continue via clinic visits through 40 weeks following the vaccine administration and through an influenza season. Refer to Section 4.6 which describes the criteria for discontinuing study participation.

### 4.4.12. Mucosal Sample Collection

Throughout the study, nasopharyngeal swabs for diagnostic purposes will be requested from all subjects who meet criteria for ILI as defined in Section 5.1.

### 4.4.13. 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.

### 4.4.14. Apheresis

Subjects in Groups 2A-2B, 3A-3B, 4A-4B, 5A-5B, and groups 6A-6B, 7A-7B, and 8A-8B (if enrolled) will be offered apheresis as an optional procedure at Visit 04 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 Section 4.4.15 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.

### 4.4.15. Apheresis Eligibility Criteria

Subject must meet all of the following criteria:

- Afebrile (temperature < 37.5°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/µL
- 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 beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) performed by a VRC study clinician within 72 hours prior to the apheresis procedure for those women with childbearing potential.

### 4.4.16. 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 other 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.

## 4.5. Criteria for Dose Escalation and Dose Continuation

There were multiple interim safety dose-escalation reviews in this study as described below. The Protocol Safety Review Team (PSRT, Section 8.7.1) conducted an interim safety data review before dose escalation occurred. The requirement was that the PSRT must assess the data as showing no significant safety concerns before enrollment of the next dose level may proceed.

In part A, enrollment began in Group 1A-1B (20 mcg of VRC-FLUMOS0111-00-VP) with no more than one subject enrolled per day for the first three subjects. When the two weeks post vaccination visit for the fifth subject in Group 1A-1B was completed, there was an interim safety review of available data which enabled the study to proceed to the next dose level.

The 20 mcg dose of VRC-FLUMOS0111-00-VP was assessed as safe and enrollment began for Group 2A-2B (60 mcg of VRC-FLUMOS0111-00-VP) with no more than one subject enrolled per day for the first three subjects only. The 60 mcg dose of VRC-FLUMOS0111-00-VP was also assessed as safe.

In Part B, enrollment began in Group 4A-4B (100 mcg of VRC-FLUMOS0111-00-VP) with no more than one subject enrolled per day for the first three subjects. When the two weeks post vaccination visit for the fifth subject in Group 4A-4B was completed, there was an interim PSRT

safety review of available data to determine whether to continue at the same dose level in Group 4A-4B and to begin enrolling Group 5A-5B. Group 5A-5B (100 mcg of VRC-FLUMOS0111-00-VP and 20% V/V of Adjuplex) began enrollment with no more than one subject enrolled per day for the first three subjects. When the two weeks post vaccination visit for the fifth subject in Group 5A-5B was completed, there was an interim safety review of available data to determine whether to continue at the same dose level. When both groups 4 and 5 were open to accrual, subjects were enrolled at the discretion of the PI in order to balance enrollments in each group for flu vaccination history, demographic considerations, and temporality as flu season progressed. An effort was be made to keep the group sizes balanced over time to ensure similar levels of exposure to influenza circulating in the community.

The optional Group 6 and Part C of the study (Groups 7-8) were not enrolled in the study.

Consultation with the IRB and FDA, if needed, as per study pause criteria (Section 4.7) will occur if indicated by the review. One outcome of a dose escalation review may be to recommend evaluation of additional subjects at the current dose level and reassess for safety before proceeding to a higher dose level and repeat dosing at the same dose level.

## 4.6. Criteria for Discontinuing Protocol Participation

All subjects will be encouraged to remain in the study and continue to receive follow-up for safety. Decisions by the PI or designee to discontinue protocol participation for a subject will be made with the following criteria.

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

## 4.7. Criteria for Pausing and Resuming the Study

### 4.7.1. Plan for Pausing the Study

The PI and Protocol Safety Review Team (PSRT) will closely monitor and analyze study data as it becomes 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.
- **One** (or more) subject experiences ulceration, abscess or necrosis at the injection site.

- **Three** (or more) subjects experience the same **Grade 2 or higher** laboratory abnormality 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.

### 4.7.2. 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 SAFETY AND ADVERSE EVENT REPORTING and institutional policy.

# 5. SAFETY AND ADVERSE EVENT REPORTING

## 5.1. Adverse Events

Adverse Event (AE) - 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. All AEs, including laboratory abnormalities, will be followed to resolution or stabilization.

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 Section 4.4.10) will be recorded without attribution assessments by the subject on paper or an electronic diary card for 7 days after 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 study injection through completion of the 4-week post-product administration visit. After that through the last study visit, only SAEs (Section 5.2), new chronic medical conditions, new diagnosed or potential autoimmune and auto-inflammatory diseases, and influenza or influenza-like illness (ILI) will be recorded.
3. Study clinicians will evaluate for cervical and axillary lymphadenopathy at every study visit through 14 days after vaccination, and if present, will assess for generalized lymphadenopathy. Lymphadenopathy will be reported as an unsolicited AE and followed to resolution through the end of the study.
4. Cases of influenza or influenza-like illness (ILI) will be evaluated as follows:

   ILI is defined per the following criteria by the CDC: fever (temperature of $100^0$F ($37.8^0$C) 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 polymerase chain reaction (PCR) per NIH CC practices in subjects 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. Reporting of Pregnancy

Pregnancy is not an adverse event, but pregnancy, if it occurs during the time of study participation, will be recorded in the study database, and notification on the pregnancy will be distributed to the study team and to the IND Sponsor. Subjects will be followed for clinical safety and to collect the pregnancy outcome as described in Section 4.4.7. Congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section 5.2) will be reported as SAEs. Pregnancy outcome will be reported to the IND Sponsor and to regulatory agencies.

## 5.5. 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 Section 8.5) 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.

## 5.6. 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

### 5.6.1. 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.

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

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

### 5.6.3. Protocol Deviation

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

- Major Deviations – Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor Deviations – Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

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

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

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

### 5.6.4. 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.

### 5.6.5. 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.

### 5.6.6. 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.

### 5.6.7. 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.

### 5.6.8. 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 the mosaic quadrivalent influenza vaccine VRC-FLUMOS0111-00-VP. Study objectives can be found in STUDY OBJECTIVES.

## 6.2. Sample Size and Accrual

In Part A, 35 healthy adult participants were enrolled in the study. In Group 1A-1B, five subjects were enrolled, and in Groups 2A-2B and 3A-3B, fifteen healthy adult participants subjects were enrolled in each group respectively.

In Part B, recruitment will target 26, and potentially 39 subjects if needed for additional safety and immunogenicity evaluation at the 60 mcg dose level (Group 6). In part C, recruitment will target 26 healthy adult participants, if needed, after analysis of Part A and B safety and immunogenicity data. Up to 110 participants may be enrolled if necessary for safety or immunogenicity evaluations.

## 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 injection and safety evaluated by clinical visits for 40 weeks following the vaccine administration and through an influenza season. See Section 4.4 and APPENDIX I: SCHEDULE OF EVALUATIONS for details and specified time points. The following endpoints will be assessed for all study groups:

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

### 6.3.2. Secondary Endpoints: Immunogenicity

**For part A**: The principal immunogenicity endpoints will be assessed by evaluation of HA specific responses to VRC-FLUMOS0111-00-VP and Flucelvax® at Week 2 (Visit 04) after vaccination.

**For part B** and **C**: The principal immunogenicity endpoints will be assessed by evaluation of HA specific responses to VRC-FLUMOS0111-00-VP alone or VRC-FLUMOS0111-00-VP with Adjuplex at Week 2 (Visit 04) after 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. Sample Size Consideration for Safety

**For part A**: Group 1A-1B (*dose-escalation*) with sample size n=5, there is over 90% chance to observe at least 1 SAE if the true rate is at least 37% and over 90% chance to observe no SAE if the true rate is no more than 2%. For Groups 2A-2B and 3A-3B with each group of size n=15, there is over 90% chance to observe at least 1 SAE if the true rate is at least 14.3% and over 90% chance to observe no SAE if the true rate is no more than 0.6%.

For **Part B** and **Part C**, with sample size n=13, there is over 90% chance to observe at least 1 SAE if the true rate is at least 16.3% and over 90% chance to observe no SAE if the true rate is no more than 0.8%.

Probabilities of observing 0 or more than 1 SAE within a group are presented in **Table 2** for a range of possible true event rates.

**Table 2: Probability of Observing Events under Different Scenarios**

| True Event Rate | n=5 in a group | | n=15 in a group | | n=13 in a group | |
|---|---|---|---|---|---|---|
| | Pr(0) | Pr(>1) | Pr(0) | Pr(>1) | Pr(0) | Pr(>1) |
| 0.005 | 0.975 | 0 | 0.928 | 0.003 | 0.937 | 0.002 |
| 0.01 | 0.951 | 0.001 | 0.86 | 0.01 | 0.878 | 0.007 |
| 0.02 | 0.904 | 0.004 | 0.739 | 0.035 | 0.769 | 0.027 |
| 0.035 | 0.837 | 0.011 | 0.586 | 0.095 | 0.629 | 0.074 |
| 0.05 | 0.774 | 0.023 | 0.463 | 0.171 | 0.513 | 0.135 |
| 0.1 | 0.59 | 0.081 | 0.206 | 0.451 | 0.254 | 0.379 |
| 0.15 | 0.444 | 0.165 | 0.087 | 0.681 | 0.121 | 0.602 |
| 0.2 | 0.328 | 0.263 | 0.035 | 0.833 | 0.055 | 0.766 |
| 0.3 | 0.168 | 0.472 | 0.005 | 0.965 | 0.01 | 0.936 |

**Table 3** gives the upper and lower bounds for 95% exact binomial confidence intervals of the true SAE rate at possible numbers of events within a group. Within a group of size n=5, if none experience an SAE, the 95% exact confidence interval has upper bound 0.522. Within a group of size n=15, if 2 enrollees experience an SAE, the exact 95% confidence interval has lower bound 0.017 and upper bound 0.405. Within a group of size n=13, if 2 enrollees experience SAE, the exact 95% confidence interval has lower bound 0.019 and upper bound 0.454.

**Table 3: 95% Confidence Intervals for the True Rate under Possible Observed Number of Events**

| Observed Number of Events | 95% Confidence Interval | 95% Confidence Interval | 95% Confidence Interval |
|---|---|---|---|

| | (n=5) | | (n=15) | | (n=13) | |
|---|---|---|---|---|---|---|
| | Lower Bound | Upper Bound | Lower Bound | Upper Bound | Lower Bound | Upper Bound |
| 0 | 0 | 0.522 | 0 | 0.218 | 0 | 0.247 |
| 1 | 0.005 | 0.716 | 0.002 | 0.319 | 0.002 | 0.36 |
| 2 | 0.053 | 0.853 | 0.017 | 0.405 | 0.019 | 0.454 |
| 3 | 0.147 | 0.947 | 0.043 | 0.481 | 0.05 | 0.538 |
| 4 | 0.284 | 0.995 | 0.078 | 0.551 | 0.091 | 0.614 |
| 5 | 0.478 | 1 | 0.118 | 0.616 | 0.139 | 0.684 |
| 6 | | | 0.163 | 0.677 | 0.192 | 0.749 |
| 7 | | | 0.213 | 0.734 | 0.251 | 0.808 |

### 6.3.5. Sample Size Consideration for Immunogenicity

Table 3 also applies to the sample size consideration for immunogenicity. Within a group of size n=15, if there are two positive responders with respect to an immunogenicity endpoint, the 95% confidence interval of the positive response rate has the lower bound of 0.017 and the upper bound of 0.405. Within a group of size n=13, if there are two positive responders with respect to an immunogenicity endpoint, the 95% confidence interval of the positive response rate has the lower bound of 0.019 and the upper bound of 0.454.

### 6.3.6. Sample Size Consideration for Comparison

For the comparison of positive response rate between two arms, we present in Table 4 the minimum event rate that can be detected with 80% or 90% power in the high-responding arm over a range of possible event rates in the low-responding arm. In **Part A** with each arm of size n=15, if the positive response rate is 0.1 in the low-responding arm, this trial has 80% (or 90%) power to detect the between-arm difference if the positive response rate in the other arm is no less than 0.57. In **Part B** and **Part C** with each arm of size n=13, if the positive response rate is 0.1 in the low-responding arm, this trial has 80% power to detect the between-arm difference if the positive response rate in the other arm is no less than 0.61.

For the comparison of the magnitude of immune response between two arms in **Part A** with each of size n=15, the trial has 80% (or 90%) power to detect the between-arm difference if the effect size is no less than 1.06 (or 1.23); that is, the mean difference is no less than 1.06 (or 1.23) the standard deviation of the immune response. In Part B and Part C with each arm of size n=13, the trial has 80% (or 90%) power to detect the between-arm difference if the effect size is no less than 1.15 (or 1.33). The power calculation is based on two-sample t-test, and a log-transformation of the immune response may be needed if the data is close to being log-normally distributed.

**Table 4: The Minimum Event Rate That Can Be Detected With 80% Or 90% Power with Sample Size n=15 and n=13 per Arm**

| Rate in one arm | Minimum rate in the other arm (80% power) | Minimum rate in the other arm (90% power) |
|---|---|---|
| 0.05 | 0.49 (0.53) | 0.56 (0.61) |

| | | |
|---|---|---|
| 0.1 | 0.57 (0.61) | 0.64 (0.68) |
| 0.2 | 0.69 (0.73) | 0.76 (0.79) |
| 0.3 | 0.79 (0.83) | 0.85 (0.88) |
| 0.4 | 0.88 (0.91) | 0.93 (0.96) |
| 0.5 | 0.95 (0.97) | 0.99 (Impossible) |
| 0.6 | 1 (Impossible) | Impossible (Impossible) |

## 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. 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.

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 by neutralization assay of samples collected 2 weeks after vaccination. 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 325 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 1** in Section 4.1.

## 7.1. Study Products

Investigational vaccine:

- VRC-FLUMOS0111-00-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 ± 18 mcg/mL in formulation buffer composed of 20mM acetate phosphate, 30 mM NaCl, 5% w/v sucrose, 2.5% w/v sorbitol, 0.01% w/v Pluronic F-68, pH 5.7. Vials are intended for single use only and do not contain preservative. Vials must not be refrozen after thawing.

Licensed Comparator QIV Flucelvax®:

- Flucelvax® is a sterile, slightly opalescent suspension in phosphate buffered saline for IM injection supplied in a 0.5 mL single-dose in a pre-filled Luer Lock syringe and is formulated to contain a total of 60 micrograms (mcg) of H1, H3, HBv, and HBy HA strains per 0.5-mL dose, in the recommended ratio of 15 mcg HA for each of the four influenza strains.
- Flucelvax® contains no adjuvants, preservatives or antibiotics. Syringes must be refrigerated at the recommended storage condition at 2°C to 8°C. Do not freeze and protect from light. Do not use after the expiration date.

Adjuvant:

- Adjuplex Adjuvant (VRC-GENADJ0110-AP-NV): is provided as 3-mL single-use glass vials with a fill volume of 0.7 mL. Each vial contains an off-white, semi-opaque (not transparent), preservative-free, sterile liquid with no visible particles and which is homogenous after a gentle shake.
- Adjuplex is mixed with vaccine in the pharmacy during preparation. prior to vaccination to maintain a 20% (v/v) dose of Adjuplex.

## 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 VRC-FLUMOS0111-00-VP and Adjuplex will have specific product and manufacturer information (e.g., product description, product number, lot number, fill volume, concentration, fill date, storage condition). Labels for the investigational products will contain an Investigational Use Statement ("Limited by Federal Law to Investigational Use").

### 7.2.2. Study Products Storage

VRC-FLUMOS0111-00-VP (FluMos-v1): 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 as this may compromise closure integrity.

Flucelvax®: 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 2°C to 8°C in a qualified, continuously monitored, temperature-controlled refrigerator. Do not freeze, protect from light and do not use after the expiration date. More information regarding QIV Flucelvax® can be found in the US Package Insert [50].

Adjuplex Adjuvant (VRC-GENADJ0110-AP-NV): Vials will be shipped within the recommended temperature range using appropriate shipping configurations to the study pharmacist or designee. Vials of adjuvant are stored until use at $2^0$C to $8^0$C in a qualified, continuously monitored, temperature-controlled refrigerator. Do not freeze.

Unopened vials may be stored for up to 24 hours at $15^0$C to $27^0$C.

### 7.2.3. Study Products Handling Information

FluMos-v1, Flucelvax®, and Adjuplex 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. The United Nation Globally Harmonized System of classification and labelling of chemicals (GHS), in accordance with 29 CFR 1910, specifies that Adjuplex causes skin irritation (H315) and serious eye irritation (H319). 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 and refrigerator, 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 for the investigational products, it must not be refrozen. Thawed vials must be quarantined at 5°C ± 3°C. For Flucelvax® vials must be quarantined at the labeled storage temperature of 2°C to 8°C. For Adjuplex vials must be quarantined at the labeled storage temperature of 2°C to 8°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 in step 3 above.
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 Products 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 VRC-FLUMOS0111-00-VP injections must be administered within 4 hours after removing the vial from the freezer. Flucelvax® single use dose injections must be administered as soon as possible after removal from the refrigerator.

The investigational study (FluMos-v1) 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.

Flucelvax® product appearance specification is a slightly opalescent suspension; 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.

Adjuplex product appearance specification notes that vials contain an off-white, semi-opaque (not transparent) liquid, with no visible particles; the liquid is homogeneous after a gentle shake.

### 7.3.1. Preparation of VRC-FLUMOS0111-00-VP for Part A Administration

Subjects received the vaccine via needle and syringe injection. The following instructions apply for Part A for VRC-FLUMOS0111-00-VP preparation.

Part A:

Group 1A-1B (*20 mcg*):

1. Thaw 1 vial of FluMos-v1 at room temperature (15º to 27º C) until all ice crystals have melted. Invert vial 3-5 times to mix.
2. Withdraw 0.11mL of FluMos-v1 using a needle sized appropriately for the dose administration; discard needle and cap the syringe for transport.

Group 2A-2B (*60 mcg*):

1. Thaw 1 vial of FluMos-v1 at room temperature (15º to 27º C) until all ice crystals have melted. Invert vial 3-5 times to mix.
2. Withdraw 0.33 mL of FluMos-v1 using a needle sized appropriately for the dose administration; discard needle and cap the syringe for transport.

### 7.3.2. Preparation of VRC-FLUMOS0111-00-VP alone or with Adjuplex for Administration:

Subjects will receive the vaccine alone or with Adjuplex via needle and syringe injection. The following instructions apply for Part B and Part C preparation.

Part B:

Group 4A-4B (*100 mcg* FluMos-v1):

1.Thaw 1 vial of FluMos-v1 at room temperature (15º to 27º C) until all ice crystals have melted. Invert vial 3-5 times to mix.

2.Withdraw 0.55 mL of FluMos-v1 using a needle sized appropriately for the dose administration; discard needle and cap the syringe for transport.

Group 5A-5B (*100 mcg* FluMos-v1 + *20% V/V* Adjuplex):

1. Thaw 1 vial of FluMos-v1 at room temperature (15º to 27º C) until all ice crystals have melted. Invert vial 3-5 times to mix.

3. Equilibrate 1 vial of Adjuplex at room temperature (15º to 27º C) for 30 minutes. Invert vial 3-5 times to mix.

4. Withdraw 0.18 mL of Adjuplex using a needle sized appropriately for the dose administration and add to the vial of FluMos-v1. Mix by inverting vial 3-5 times.

5. Withdraw 0.70 mL for administration.

Group 6A-6B (*60 mcg* FluMos-v1 + *20% V/V* Adjuplex):

1. Thaw 1 vial of FluMos-v1 at room temperature (15º to 27º C) until all ice crystals have melted. Invert vial 3-5 times to mix.

2. Equilibrate 1 vial of Adjuplex at room temperature (15º to 27º C) for 30 minutes. Invert vial 3-5 times to mix.

3. Withdraw 0.18 mL of Adjuplex using a needle sized appropriately for the dose administration and add to the vial of FluMos-v1. Mix by inverting vial 3-5 times.

4. Withdraw 0.42 mL for administration

From in-use stability report: Prepared syringes may be stored at 5°C ± 3°C for up to 7 hours or at ambient temperatures (maximum 27°C) for a maximum of 4 hours (include administration time).

Part C

Group 7A-7B (*TBD mcg* FluMos-v1):

TBD

Group 8A-8B (*TBD mcg* FluMos-v1 + *20% V/V* Adjuplex):

TBD

### 7.3.3. Preparation of Flucelvax® for Administration:

Group 3A-3B (Flucelvax®)

Subjects received the vaccine via needle and syringe injection. Administer Flucelvax® as a single 0.5 mL intramuscular injection in the region of the deltoid muscle of the upper arm. Do not inject the vaccine in the gluteal region or areas where there may be a major nerve trunk. Flucelvax® should not be administered intravascularly, intradermally or subcutaneously. Do not use the vaccine if the contents have been frozen. Flucelvax® should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit, if either condition exists, do not administer the vaccine.

3. Take the 0.5 ml single dose pre-filled Luer Lock Syringe out of the fridge
4. Shake the syringe vigorously before administration
5. Attach a sterile needle to the pre-filled syringe and administer intramuscularly.

### 7.3.4. Administration of Injections

Clinician instructions on how to select an arm and administer an injection are in Section 4.4.8. Subjects will receive the vaccine by needle and syringe injection in either deltoid. Clinicians will choose the appropriate needle size for each subject. The syringe should be inverted 5x to mix before product administration. 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 Section 5.6.

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-FLUMOS0111-00-VP

VRC 325 is the first study in humans of the investigational vaccine, FluMos-v1, VRCFLUMOS0111- 00-VP, and so there is limited data beyond that summarized above. The risks described below are based on the existing data with FluMos-v1, risks of vaccines in general, and results of previous studies with investigational VRC vaccines.

Side effects seen with FluMos-v1 as detailed in Section 1.5.1 include pain and tenderness, redness of the skin, pruritus, and swelling at the injection site. Systemic symptoms include malaise, headache, chills, myalgia, nausea, fever, and joint pain. Other potential side effects include cervical lymphadenopathy, transient neutropenia, and transient lymphopenia.

Other potential side effects resulting from intramuscular injection include stinging, arm discomfort, mild bruising, or a small laceration at the vaccine injection site.

Subjects may exhibit other general signs and symptoms associated with administration of a vaccine injection, including rash, aches and pains, and dizziness. These side effects should be monitored but are generally of short duration, mild to moderate severity, and usually do not require treatment. Allergic reactions can also be seen following vaccinations with and without adjuvants, which can include severe allergic reactions like anaphylaxis.

There is no knowledge regarding the possible effects of the study vaccine on the fetus or nursing infant.

### 8.5.2. Risks of Flucelvax®

The following signs and symptoms have been associated with the administration of previous Flucelvax® seasonal vaccines in adults 18 to 64 years as discussed in Sections 1.5.5 and 1.6.3. Subjects have reported pain at the injection site, erythema at the injection site, headache, fatigue, myalgia, and malaise within 7 days after vaccination with Flucelvax®. The most reported unsolicited adverse events were rhinitis and oropharyngeal pain after 21 days of vaccination with Flucelvax®.

The following adverse events were reported voluntarily to the FDA by subjects during post-approval use of Flucelvax®: immune system disorders (anaphylactic reaction, angioedema), skin and subcutaneous tissue disorders (generalized skin reactions including pruritus, urticaria or non-specific rash), nervous systems disorders (Syncope, presyncope, paresthesia), and general disorders and administration site conditions (extensive swelling of the injected limbs); however, it is not always possible to evaluate a reliably estimate of their frequency or establish a causal relationship to the vaccine.

### 8.5.3. Risks of Adjuplex

Adjuplex is a novel adjuvant platform that has been evaluated in a limited number of clinical trials, and therefore, data on risks remain limited.

Potential inflammatory reactions at the injection site may be expected, similar to the injection site reactions observed with other adjuvants. This can manifest as mild to severe pain, redness,

and swelling at the site of administration. When combined with a vaccine, Adjuplex may cause more frequent or severe systemic reactions, such as fever, chills, myalgia, or malaise, compared to formulations containing the vaccine only. Rash, urticaria, and transient elevation of the absolute eosinophil count, and one event of serum sickness have been observed after administration of an Adjuplex-adjuvanted HIV vaccine.

Soy lecithin is one of the components of Adjuplex. Highly-purified soy lecithin, as used in Adjuplex, does not contain soy protein residues that are associated with soy allergy [55, 56]

Since the experience with Adjuplex in humans is limited, there may be other unknown side effects.

### 8.5.4. 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.
- *Mucosal sampling*: Collection of samples by nasopharyngeal or oral swabs rubbed over the mucosal surfaces may cause momentary discomfort and, in some cases, minor bleeding.

### 8.5.5. Risks of Study Vaccine for 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 study injection and asked to notify the site immediately upon learning of a pregnancy during this study. In the case of pregnancy, subjects 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.

### 8.5.6. 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.

### 8.5.7. Risks of Screening Procedures

The risks of screening procedures can be found in the VRC's screening protocol, VRC 500 (NIH 11-I-0164, NCT01375530) used for all VRC IND studies conducted at the NIH Clinical Center.

### 8.5.8. 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 a Flu mosaic (or universal) influenza virus vaccine. The investigational vaccine is not expected to provide protection from influenza.

### 8.5.9. Assessment of Potential Risks and Benefits

This healthy volunteer trial to evaluate the safety and immunogenicity of FluMos-v1 was reviewed using the VRC Risk Management Plan. Potential risks, acceptance of risks, and mitigation strategies are available in the VRC 325 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 for exploratory genetic factors that may influence the immune response to vaccination. For example, this could include RNA transcriptome, immunoglobulin genes, and polymorphisms, in pattern recognition receptors (PRRs), such as Toll-like receptor (TLR) or RIG-like receptor (RLR) genes. In addition, it could include many single-nucleotide polymorphisms (SNPs) in other genes, for example, those coding for cytokines, viral, or vitamin receptors that are experimentally associated with variations in vaccine responses. No specific results will be provided to participants or their health care providers because we will not be investigating genetic analyses that have known medical diagnoses (e.g. Huntington's disease) or other medically actionable genetic information.

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 is comprised of the PI, Associate Investigators, Study Coordinator, Protocol Specialists, other Study Clinicians, and the IND Medical Officer (MO). In addition, an Independent Safety Monitor, who is not associated with the VRC, will provide safety oversight. PSRT will review the summary study safety data reports on a weekly basis through 4 weeks after the last subject receives the final study injection. 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 data, records, and other information associated with and concerning the subject. 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, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The Principal Investigator will provide direct access to all trial related 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 launches new global influenza strategy*. 2019; Available from: https://www.who.int/news-room/detail/11-03-2019-who-launches-new-global-influenza-strategy.

2. CDC. *2019-2020 U.S. Flu Season: Preliminary In-Season Burden Estimates*. 2020 04APR2020; Available from: https://www.cdc.gov/flu/about/burden/preliminary-in-season-estimates.htm.

3. Mao, L., et al., *Annual economic impacts of seasonal influenza on US counties: spatial heterogeneity and patterns.* Int J Health Geogr, 2012. 11: p. 16.

4. Molinari, N.A., et al., *The annual impact of seasonal influenza in the US: measuring disease burden and costs.* Vaccine, 2007. 25(27): p. 5086-96.

5. Luke, C. and K. Subbarao, *Vaccines for pandemic influenza.* Emerging Infectious Diseases, 2006. 12(1): p. 66-72.

6. Kapoorm, S. and K. Dhama, *Insight into Influenza Viruses of Animals and Humans*. 2014, Springer: Springer. 230.

7. Tennant, P., G. Fermin, and J.E. Foster, *Viruses. Molecular Biology, Host Interactions and Applications to Biotechnology*. 2018: Elsevier Inc. 392.

8. Taubenberger, J.K. and J.C. Kash, *Influenza virus evolution, host adaptation, and pandemic formation.* Cell Host Microbe, 2010. 7(6): p. 440-51.

9. Laursen, N.S. and I.A. Wilson, *Broadly neutralizing antibodies against influenza viruses.* Antiviral research, 2013. 98(3): p. 476-483.

10. 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.

11. Ekiert, D.C., et al., *Antibody recognition of a highly conserved influenza virus epitope.* Science, 2009. 324(5924): p. 246-51.

12. 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.

13. 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.

14. 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.

15. CDC. *Types of Influenza Viruses*. 18NOV2019; Available from: https://www.cdc.gov/flu/about/viruses/types.htm.

16. Medina, R.A. and A. Garcia-Sastre, *Influenza A viruses: new research developments.* Nat Rev Microbiol, 2011. 9(8): p. 590-603.

17. Tripp, R.A. and S.M. Tompkins, *Virus-vectored influenza virus vaccines.* Viruses, 2014. 6(8): p. 3055-79.

18. CDC. *Seasonal Flu Vaccine Effectiveness Studies*. 2020 01JUL2020; Available from: https://www.cdc.gov/flu/vaccines-work/effectiveness-studies.htm.

19. Sharma, L., A. Rebaza, and C.S. Dela Cruz, *When "B" becomes "A": the emerging threat of influenza B virus.* European Respiratory Journal, 2019. 54(2): p. 1901325.

20. Barr, I.G., et al., *Cell culture-derived influenza vaccines in the severe 2017-2018 epidemic season: a step towards improved influenza vaccine effectiveness.* NPJ Vaccines, 2018. 3: p. 44.

21. Lambert, L.C. and A.S. Fauci, *Influenza vaccines for the future.* N Engl J Med, 2010. 363(21): p. 2036-44.

22. Krammer, F. and P. Palese, *Advances in the development of influenza virus vaccines.* Nat Rev Drug Discov, 2015. 14(3): p. 167-82.

23. Adam D. DeZure, et al., *Safety and Immunogenicity of H7 Influenza Prime-Boost Regimens in Healthy Adults*, in *National Foundation for Infectious Diseases*. 2016: Baltimore, MD.

24. Boyoglu-Barnum, S., et al., *Elicitation of broadly protective immunity to influenza by multivalent hemagglutinin nanoparticle vaccines.* bioRxiv, 2020: p. 2020.05.30.125179.

25. Fischer, M., et al., *Evolution of vitamin B2 biosynthesis. A novel class of riboflavin synthase in Archaea.* J Mol Biol, 2004. 343(1): p. 267-78.

26. Zylberman, V., et al., *Evolution of vitamin B2 biosynthesis: 6,7-dimethyl-8-ribityllumazine synthases of Brucella.* J Bacteriol, 2006. 188(17): p. 6135-42.

27. Jardine, J., et al., *Rational HIV immunogen design to target specific germline B cell receptors.* Science, 2013. 340(6133): p. 711-6.

28. Ueda, G., et al., *Tailored design of protein nanoparticle scaffolds for multivalent presentation of viral glycoprotein antigens.* Elife, 2020. 9.

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

30. 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).

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

32. Romanova, J., *Influenza vaccines manufacturing in continuous cell lines: problems and solutions.* MIR Journal, 2017. 4: p. 1-9.

33. Rajaram, S., et al., *Influenza vaccines: the potential benefits of cell-culture isolation and manufacturing.* Therapeutic advances in vaccines and immunotherapy, 2020. 8: p. 2515135520908121-2515135520908121.

34. Bernstein, D.I., *Cell culture–derived influenza vaccines: has their time come?* Clin Infect Dis, 2010. 51(9): p. 1005-6.

35. Kon, T.C., et al., *Influenza Vaccine Manufacturing: Effect of Inactivation, Splitting and Site of Manufacturing. Comparison of Influenza Vaccine Production Processes.* PLoS One, 2016. 11(3): p. e0150700.

36. Gasper, D.J., et al., *Effective Respiratory CD8 T-Cell Immunity to Influenza Virus Induced by Intranasal Carbomer-Lecithin-Adjuvanted Non-replicating Vaccines.* PLoS Pathog, 2016. 12(12): p. e1006064.

37. Lee, W., et al., *Carbomer-based adjuvant elicits CD8 T-cell immunity by inducing a distinct metabolic state in cross-presenting dendritic cells.* PLoS Pathog, 2021. 17(1): p. e1009168.

38. Lee, W., et al., *Combination Adjuvants Affect the Magnitude of Effector-Like Memory CD8 T Cells and Protection against Listeriosis.* Infect Immun, 2021. 89(7): p. e0076820.

39. Kong, R., et al., *Antibody Lineages with Vaccine-Induced Antigen-Binding Hotspots Develop Broad HIV Neutralization.* Cell, 2019. 178(3): p. 567-584 e19.

40. Wegmann, F., et al., *The Carbomer-Lecithin Adjuvant Adjuplex Has Potent Immunoactivating Properties and Elicits Protective Adaptive Immunity against Influenza Virus Challenge in Mice.* Clin Vaccine Immunol, 2015. 22(9): p. 1004-12.

41. Sastry, M., et al., *Adjuvants and the vaccine response to the DS-Cav1-stabilized fusion glycoprotein of respiratory syncytial virus.* PLoS One, 2017. 12(10): p. e0186854.

42. Ou, L., et al., *Preclinical Development of a Fusion Peptide Conjugate as an HIV Vaccine Immunogen.* Sci Rep, 2020. 10(1): p. 3032.

43. Cheng, C., et al., *Immune Monitoring Reveals Fusion Peptide Priming to Imprint Cross-Clade HIV-Neutralizing Responses with a Characteristic Early B Cell Signature.* Cell Rep, 2020. 32(5): p. 107981.

44. Zhou, T., et al., *Quantification of the Impact of the HIV-1-Glycan Shield on Antibody Elicitation.* Cell Rep, 2017. 19(4): p. 719-732.

45. Havlicek, D.F., et al., *Cocaine vaccine dAd5GNE protects against moderate daily and high-dose "binge" cocaine use.* PLoS One, 2020. 15(11): p. e0239780.

46. Hicks, M.J., et al., *Fate of systemically administered cocaine in nonhuman primates treated with the dAd5GNE anticocaine vaccine.* Hum Gene Ther Clin Dev, 2014. 25(1): p. 40-9.

47. Maoz, A., et al., *Adenovirus capsid-based anti-cocaine vaccine prevents cocaine from binding to the nonhuman primate CNS dopamine transporter.* Neuropsychopharmacology, 2013. 38(11): p. 2170-8.

48. Houser, K.V., et al., *Safety and immunogenicity of a ferritin nanoparticle H2 influenza vaccine in healthy adults: a phase 1 trial.* Nat Med, 2022. 28(2): p. 383-391.

49. Widge, A.T., et al., *An influenza hemagglutinin stem nanoparticle vaccine induces cross-group 1 neutralizing antibodies in healthy adults.* Sci Transl Med, 2023. 15(692): p. eade4790.

50. FDA. *US Package Insert for FLUCELVAX*. 2016 11/12/19; Available from: https://www.fda.gov/media/85322/download.

51. Frey, S., et al., *Clinical efficacy of cell culture–derived and egg-derived inactivated subunit influenza vaccines in healthy adults.* Clin Infect Dis, 2010. 51(9): p. 997-1004.

52. Szymczakiewicz-Multanowska, A., et al., *Safety and immunogenicity of a novel influenza subunit vaccine produced in mammalian cell culture.* J Infect Dis, 2009. 200(6): p. 841-8.

53. Reisinger, K.S., et al., *Subunit influenza vaccines produced from cell culture or in embryonated chicken eggs: comparison of safety, reactogenicity, and immunogenicity.* J Infect Dis, 2009. 200(6): p. 849-57.

54. Xu, K., et al., *Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1.* Nat Med, 2018. 24(6): p. 857-867.

55. Ueberham, E., et al., *Simplified Tracking of a Soy Allergen in Processed Food Using a Monoclonal Antibody-Based Sandwich ELISA Targeting the Soybean 2S Albumin Gly m 8.* Journal of Agricultural and Food Chemistry, 2019. 67(31): p. 8660-8667.

56. Taylor, S.L. and J.L. Baumert. *Allergenicity of Soy Lecithin*. 2017; Available from: https://farrp.unl.edu/documents/OpinionsSummaries/2017_0928_Soy%20Lecithin.pdf.

# APPENDIX I: SCHEDULE OF EVALUATIONS

| | | VRC 500 | VRC 325 Schedule of Evaluations for Part A | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | | *01 | 02 | 02A | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 |
| **Week of Study** | | -8 to 0 | W0 | W1 | W1 | W2 | W4 | W12 | W16 | W22 | W28 | W40 |
| **Day of Study** | | -56 to 0 | [1]D0 | D1 | D6 | D14 | D28 | D84 | D112 | D154 | D196 | D280 |
| **Clinical** | Tube | | | | | | | | | | | |
| *VRC 500 Screening Consent | | X | | | | | | | | | | |
| VRC 325 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 | | X | X | | X | X | X | X | X | X | X | X |
| [3]Study Product Administration:<br>**Part A:**<br>**Group 1A-1B: FluMos-v1 (20 mcg)**<br>**Group 2A-2B: FluMos-v1 (60 mcg)**<br>**Group 3A-3B: Flucelvax® (60 mcg)** | | | **X** | | | | | | | | | |
| Phone evaluation (clinic visit as needed) | | | | X | | | | | | | | |
| Begin diary card | | | X | | | | | | | | | |
| [4]Pregnancy test: urine or serum | | X | X | | | [8](X) | | | | | | X |
| [4]Pregnancy prevention counseling/Reproductive Information Form | | X | X | | | [8](X) | | | | | | X |
| CBC with differential | EDTA | 3 | 3 | | | 3 | 3 | | | | | 3 |
| 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** | | | | | | | | | | | | |
| *[9]Oral Mucosal sample collection for antibody analysis* | SST | | X | | | X | X | | X | | | |
| *Engineered pentameric scaffold lumazine synthase and engineered trimeric domain antibody analysis* | SST | | X | | | X | | | X | | | |
| *Serum* | SST | 16 | 16 | | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| *PBMC and plasma* | EDTA | 40 | 80 | | [6]80 | [7]120 or Apheresis (6) | 80 | 80 | 80 | 40 | 80 | 80 |
| **Daily Volume (mL)** | | 66 | 103 | 0 | 96 | 143 | 103 | 96 | 96 | 56 | 96 | 103 |
| **Max. Cumulative Volume (mL)** | | 66 | 169 | 169 | 265 | 408 | 511 | 607 | 703 | 759 | 855 | 958 |

* 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 may have occurred since screening, then physical examination & laboratory studies done on Day 0 are used for eligibility.

1 Day 0=day of enrollment and vaccine injection. Day 0 evaluations prior to 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 Study Product Administration: 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 study injection.
5 A nasopharyngeal swab for the SARS-CoV-2 PCR should be collected no more than 5 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.
7 Only for subjects in Groups 2A-2B and 3A-3B: If optional Apheresis occurs, ONLY draw 16 mL in SST (DO NOT draw 120 ml in EDTA tubes). Group 1A-1B does not have a large blood draw or optional apheresis. Only for subjects in Group 1A-1B: draw 16 mL in SST and 80 mL in EDTA.
8 For women of reproductive potential, pregnancy test must be negative within 72 hours prior to apheresis procedure.
9 Only for subjects in Groups 2A-2B and 3A-3B. There are two oral buccal samples.

| | | VRC 500 | VRC 325 Schedule of Evaluations for Part B and Part C | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | | *01 | 02 | 02A | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 |
| **Week of Study** | | -8 to 0 | W0 | W1 | W1 | W2 | W4 | W12 | W16 | W22 | W28 | W40 |
| **Day of Study** | | -56 to 0 | [1]D0 | D1 | D6 | D14 | D28 | D84 | D112 | D154 | D196 | D280 |
| **Clinical** | Tube | | | | | | | | | | | |
| *VRC 500 Screening Consent | | X | | | | | | | | | | |
| VRC 325 AoU; Consent | | | X | | | | | | | | | |
| [2]Physical exam for eligibility, BMI, vitals at screening; BMI, vital signs and targeted exam as needed at baseline; vital signs and targeted exam as needed at other visits. | | X | X | | X | X | X | X | X | X | X | X |
| Medical history targeted to eligibility at screening; then interim medical history | | X | X | | X | X | X | X | X | X | X | X |
| [3]Study Product Administration:<br>**Part B:**<br>**Group 4A-4B: FluMos-v1 (100 mcg)**<br>**Group 5A-5B: FluMos-v1 (100 mcg) + Adjuplex**<br>**Group 6A-6B: FluMos-v1 (60 mcg) + Adjuplex**<br>**Part C:**<br>**Group 7A-7B: FluMos-v1 (X mcg)**<br>**Group 8A-8B: FluMos-v1 (X mcg) + Adjuplex** | | | **X** | | | | | | | | | |
| Phone evaluation (clinic visit as needed) | | | | X | | | | | | | | |
| Begin diary card | | | X | | | | | | | | | |
| [4]Pregnancy test: urine or serum | | X | X | | | [7](X) | | | | | | X |
| [4]Pregnancy prevention counseling/Reproductive Information Form | | X | X | | | [7](X) | | | | | | X |
| CBC with differential | EDTA | 3 | 3 | | | 3 | 3 | | | | | 3 |
| Total bilirubin, AST, ALT, and ALP | GLT | 4 | 4 | | | 4 | 4 | | | | | 4 |
| Creatinine | | X | X | | | X | | | | | | |
| HIV (other tests, if needed) | EDTA | 3 | | | | | | | | | | |
| **Research Samples** | | | | | | | | | | | | |
| *Engineered pentameric scaffold lumazine synthase and engineered trimeric domain antibody analysis* | SST | | X | | | X | | | X | | | |
| *Serum* | SST | | 24 | | | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| *PBMC and plasma* | EDTA | | 140 | | [5]80 | [6]120 or Apheresis (6) | 80 | 80 | 80 | 40 | 80 | 80 |
| **Daily Volume (mL)** | | 10 | 171 | 0 | 80 | 143 | 103 | 96 | 96 | 56 | 96 | 103 |
| **Max. Cumulative Volume (mL)** | | **10** | **181** | **181** | **261** | **404** | **507** | **603** | **699** | **755** | **851** | **954** |

Visit windows: Schedule Visits 02A - 10 with respect to Day 0 per the following visit windows:
Visit 02A (+1 day). Visit 03 (±1 day). Visits 04 (-2/+7), 05 (±2 days). Visits 06, 07, 08, 09, 10 (±7 days).

* 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 may have occurred since screening, then physical examination & laboratory studies done on Day 0 are used for eligibility.
1 Day 0=day of enrollment and vaccine injection. Day 0 evaluations prior to injection are the baseline for assessing adverse events subsequently.
2 Physical exam for eligibility, BMI and vital signs at screening; BMI, vital signs at baseline and targeted exam as needed; vital signs at all other visits with targeted exam as needed
3 Study Product Administration: Complete post vaccination evaluations (BP, pulse, temperature, respiratory rate 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 study injection.
5 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.
6 For all groups except for Group 1: If optional Apheresis occurs, ONLY draw 16 mL in SST (DO NOT draw 120 ml in EDTA tubes).
Group 1A-1B does not have a large blood draw or optional apheresis. Only for subjects in Group 1A-1B: draw 16 mL in SST and 80 mL in EDTA.
7 For women of reproductive potential, pregnancy test must be negative within 72 hours prior to apheresis procedure.

# 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 |
| | | | | |

| [4] **Vital Signs** | **Mild (Grade 1)** | **Moderate (Grade 2)** | **Severe (Grade 3)** | **Potentially Life Threatening (Grade 4)** |
|---|---|---|---|---|
| [5]Fever (°C)<br>(°F) | 38.0 – 38.4<br>100.4 – 101.1 | 38.5 – 38.9<br>101.2 – 102.0 | 39.0 – 40<br>102.1 – 104 | > 40<br>> 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]Bruising or skin lesion associated with study injection will be assessed using the same severity grading as for erythema/redness.
[3]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<br>Fasting – mg/dL<br>Random – mg/dL | <br>100 – 110<br>110 – 125 | <br>111 – 125<br>126 – 200 | <br>>125<br>>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.

**PRINCIPAL INVESTIGATOR:** Maxwell Norris, MD

**STUDY TITLE:** VRC 325 (000410): A Phase I Open-Label Clinical Trial to Evaluate the Dose, Safety, Tolerability and Immunogenicity of Mosaic Quadrivalent Influenza Vaccine Compared with a Licensed Inactivated Seasonal QIV, In Healthy Adults

**STUDY SITE:** NIH / NIAID / VRC / Vaccine Evaluation Clinic (VEC)

Cohort: *Healthy volunteer*

Consent Version: January 10, 2024 (Version 5.0)

## WHO DO YOU CONTACT ABOUT THIS STUDY?

Principal Investigator: Maxwell Norris, MD; [redacted]

Study Coordinator: Floreliz Mendoza, RN; [redacted]

## KEY INFORMATION ABOUT THIS RESEARCH

This consent form describes a research study and is designed to help you decide if you would like to be a part of the research study.

You are being asked to take part in a research study at the National Institutes of Health (NIH). This section provides the information we believe is most helpful and important to you in making your decision about participating in this study. Additional information that may help you decide can be found in other sections of the document. Taking part in research at the NIH is your choice.

This is a study of an experimental vaccine called the FluMos-v1 vaccine for prevention of seasonal influenza (flu). The main purpose of this study is to see if the experimental vaccine alone or with adjuvant is safe and how your body responds to it. We also want to study immune responses to this vaccine compared to the 2020-2021 FDA-approved seasonal flu vaccine, Flucelvax®.

Since this is the first time that the FluMos-v1 vaccine alone or with adjuvant will be given to people, we do not know how your body will respond. This study is not designed to protect you from the flu.

This study has 3 parts: In **Part A**, 35 people already took part and got a vaccination with either FluMos-v1 or Flucelvax®. In **Part B** and **Part C**, about 65 people may get FluMos-v1 vaccine alone or with an adjuvant, depending on what group they are in. All study visits will occur at the NIH Clinical Center in Bethesda, MD.

You will be in the study for about 40 weeks (10 months) and will have about 10 clinic visits. During the study, we will collect blood samples from you, about 6-19 tubes of blood may be drawn at your visits. Some of your blood will be used to check your health and some will be

stored for future research. You will be compensated for your time and efforts for taking part in this study.

You will get the experimental FluMos-v1 vaccine or FluMos-v1 with adjuvant by injections (shots) in the upper arm muscle. This is called an intramuscular "IM" injection. We will use a needle and syringe to give you the FluMos-v1 or FluMos-v1 with adjuvant.

You could have side effects from the FluMos-v1 or adjuvant, such as fever, tiredness, body aches, headache, chills, nausea, and joint pain. These side effects can also occur with FDA-approved flu vaccines and adjuvants. The side effects usually occur within the first 24 hours after the vaccine and adjuvants are given. Rarely, side effects of trouble breathing, itchiness, rash, hives, swelling, or chest pain may occur. Some vaccines and adjuvants have a risk of serious allergic reactions that can be life threatening.

We do not know how the experimental vaccines or adjuvant may affect a fetus or nursing infant. Therefore, women who can become pregnant must have a negative pregnancy test before the injection and agree to use effective birth control beginning at least 21 days before the injection until the end of the study.

The remaining document will now describe the research study in more detail. This information should be considered before you make your choice. Members of the study team will talk with you about the information in this document. Some people have personal, religious, or ethical beliefs that may limit the kinds of medical or research interventions in which they would want to participate. Take the time you need to ask any questions and discuss this study with NIH staff, and with your family, friends, and personal health care providers.

## IT IS YOUR CHOICE TO TAKE PART IN THE STUDY

You may choose not to take part in this study for any reason. If you join this study, you may change your mind and stop participating in the study at any time and for any reason. In either case, you will not lose any benefits to which you are otherwise entitled. However, to be seen at the NIH, you must be taking part in a study or are being considered for a study. If you do choose to leave the study, please inform your study team to ensure a safe withdrawal from the research.

## WHY IS THIS STUDY BEING DONE?

Influenza (flu) is a contagious respiratory illness caused by influenza viruses that infect the nose, throat, and sometimes the lungs. It can cause mild to severe illness. Serious outcomes of flu infection can result in hospitalization or death. Some people, such as older people, young children, and people with certain health conditions, are at higher risk of serious flu complications.

This is the first study of this experimental vaccine in people. "Experimental" means that the study vaccine has not been approved by the Food and Drug Administration (FDA). The FDA allows this vaccine to be used for research purposes only.

Vaccines are given to teach the body to prevent or fight an infection. In this study, we are testing one experimental vaccine that was developed by the Vaccine Research Center (VRC) at the NIH called FluMos-v1. This vaccine is intended to help the body to make an immune response to the seasonal flu.

Most vaccines are made of proteins that are injected into a muscle. Proteins are natural substances that the body uses as building blocks. This vaccine is made in the laboratory with five proteins: four proteins similar to the seasonal influenza and one protein called lumazine synthase, which helps make vitamin B2 in the yeast *Candida albicans (C. albicans*). These five proteins have been modified in the laboratory. When combined, they make a particle that looks like the outside of the seasonal flu viruses. The body's immune system may respond to this particle.

Adjuvants are ingredients used in some vaccines that helps create a stronger immune response in people receiving the vaccine. In other words, adjuvants help vaccines work better. In this study, we are testing one experimental adjuvant: Adjuplex. This adjuvant is intended to help the body to make a stronger immune response to the FluMos-v1 vaccine.

**This is the first study to give the FluMos-v1 vaccine to humans**. We do not know if the FluMos-v1 vaccine will protect you from flu. There is no virus or yeast in the vaccine, so you cannot get an influenza or *C. albicans* infection from this vaccine.

The purpose of this research study is to see if the FluMos-v1 vaccine alone or with adjuvant is safe and how your body responds to it. We will tell you if we learn anything new during this study that might cause you to change your mind about staying in the study. At the end of the study, we will tell you when study results may be available and how to learn about them.

## WHAT WILL HAPPEN DURING THE STUDY?

This study has 3 parts as shown in the Study Design table below. In **Part A**, Groups 1A-1B and 2A-2B got different doses of FluMos-v1 while Group 3A-3B got the Flucelvax® vaccine. In **Part B** and **Part C,** new participants will get the experimental FluMos-v1 vaccine alone or with adjuvant.

The table below shows the study plan:

| VRC 325 Vaccination Schema | | | |
|---|---|---|---|
| **Group** | **Subjects** | **Day 0** | **Product** |
| **Part A** | | | |
| 1A-1B | **5** | 20 micrograms | FluMos-v1 |
| 2A-2B | **15** | 60 micrograms | FluMos-v1 |
| 3A-3B | **15** | 60 micrograms | Flucelvax® |
| **Part B** | | | |
| 4A<br>4B* | **13** | 100 micrograms | FluMos-v1 |
| 5A<br>5B* | **13** | 100 micrograms | FluMos-v1 + Adjuplex |
| 6A<br>6B* | **13** | 60 micrograms | FluMos-v1 + Adjuplex |
| **Part C** | | | |
| 7A<br>7B* | **13** | X micrograms | FluMos-v1 |
| 8A<br>8B* | **13** | X micrograms | FluMos-v1 + Adjuplex |
| **Total** | **100** | *Includes participants who got the 2021-2022 or 2022-2023 seasonal influenza vaccine.<br>Groups 6, 7, and 8 are optional. | |

If you are a woman who can get pregnant, we will do a pregnancy test before your vaccination. The test must show that you are not pregnant before you can get the vaccine.

**Follow-Up after Vaccination**

You will need to stay in the clinic for at least 30 minutes after vaccination. If you are unwell or have ongoing symptoms, you will be asked to stay in the clinic until evaluation and discharge by a study clinician. This includes the possibility of an overnight inpatient stay to evaluate for safety.

In keeping with the NIH Clinical Center 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.

The day after your vaccination, clinic staff will call to check on you. Also, after your vaccination, you will need to complete a diary card for 7 days. On the diary card you will need to record any symptoms that you may have for data analysis and not because of any risk of getting the flu from this vaccine. We will give you a thermometer to check your temperature every day for 7 days, even if you feel well. We will also give you a ruler to measure any skin changes at the injection site. You will get a password to a secure website where you can enter this data online. If you do not have access to the internet, you can use a paper diary card instead.

If you have any symptoms or feel unwell, you should tell a clinic nurse or doctor as soon as possible. You can reach the staff by phone 24 hours a day. If you have symptoms, you may be asked to come to the clinic for a checkup. It is very important that you follow the instructions from the clinic staff.

Follow-up visits allow us to check you for any health changes or problems. We will ask you how you are feeling and if you have taken any medications. We will take about 6-19 tubes of blood at each visit for safety and/or research tests. Blood draw volumes will be within NIH Clinical Center limits. We will tell you right away if any of the clinical laboratory test results show a health problem. You might need to have extra clinic visits or laboratory tests if you have health changes that need to be checked.

Clinical studies follow a set schedule. **It is important that you follow the schedule as closely as possible**. **You should try to not miss any visits.** You should contact the clinic staff as soon as possible if you need to change the date or time of any study visit.

**Collection of Blood**: We will draw your blood before your vaccination and at each scheduled follow-up clinic visit. Some of the blood that we take will be used for research and some will be used so we can monitor your health throughout the study.

For all subjects we will collect 6 to 19 tubes or 4 to 12 tablespoons of blood from you at each study visit. The total amount of blood drawn from you during the entire 40 week study will be about 954 mL. However, we will not draw more than 550 mL in any 8 week period as per NIH Clinical Center guidelines.

**Collection of Nose and Throat Secretions for Diagnosis**: If you are not feeling well and have any of the following symptoms at any time during the study, it is very important for you to contact the clinic. You may have a flu-like illness or the flu. Symptoms include:

- fever of $100^0$ F or higher,
- runny nose,
- sore throat,
- headache,
- feeling more tired than usual
- muscle aches.

If you have any of these symptoms, you will need to come to the clinic so that we can swab your nose and throat to test for the flu and other common causes. We will use a thin disposable swab to for this test.

**Apheresis**: We would like to collect your blood one time by a method called "apheresis" at 2 weeks after your study injection. This procedure is optional and choosing not to take part will not affect your study participation.

To be eligible for apheresis, <u>you must not</u>:

- have an unstable heart as indicated by your medical history and test results
- have blood pressure greater than 180/100
- have a known blood clotting disorder
- be pregnant or breast feeding
- have a condition that the attending physician or the apheresis clinic staff considers a reason to not do an apheresis procedure.

Before apheresis, we will check your weight, pulse and blood pressure. We will ask questions about your general health and medical history. If you are a woman who can get pregnant, we will do a pregnancy test before the apheresis procedure. The test must show that you are not pregnant. The apheresis staff will prick your finger to test your blood for anemia before the procedure.

During the procedure, you will lay on a recliner, couch, or hospital bed. A sterile needle will be placed into a vein in both of your arms. The kits used to collect apheresis samples are sterile, single-use, disposable sets that are not in contact with any person's bodily fluids other than yours. No blood products are given to you during these procedures. Apheresis is done at the NIH Clinical Center, and a physician from the NIH Department of Transfusion Medicine will be available in or near the apheresis area at all times.

In the apheresis procedure, blood is removed through a needle in the vein of one arm, spun in a machine that separates the white blood cells and then the rest of your blood is returned to you through a needle in the other arm. A medication called Citrate, is added to the blood while in the machine to prevent your blood from clotting.

The purpose of this procedure is to allow us to get a large number of white blood cells that cannot be collected by simple blood drawing. We want to study these white blood cells. The number of white blood cells collected is a small fraction of the total amount in your body. The body quickly replaces the cells that have been removed. The NIH Blood Bank at the Clinical Center and other blood banks use similar procedures every day to collect blood samples from donors. We will not use your samples for transfusion or therapy. The procedure will take approximately 3-4 hours.

## MONITORING OF THE STUDY

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

## GENETIC TESTING

Some of the blood drawn from you during this study will be used for genetic tests. Some genetic tests are done in research studies to see if there are genetic difference in immune responses. Your blood sample used in these genetic tests will not have your name on it, and the results will not be in your medical record.

## HOW LONG WILL THE STUDY TAKE?

If you agree to take part in the study, you will have 1 vaccination visit, 1 phone call follow-up, and 8 follow-up clinic visits over 40 weeks.

The vaccination visit will take about 6 hours. Most other follow-up clinic visits will take about 1 to 2 hours; the optional apheresis visit will take 3 to 4 hours.

## HOW MANY PEOPLE WILL PARTICIPATE IN THIS STUDY?

In **Part A**, 35 people took part. In **Part B**, we plan to enroll 39 people. This includes about 13 people who will get the 100 mcg dose of FluMos-v1 vaccine, 13 people who will get the 100 mcg dose of FluMos-v1 vaccine with adjuvant, and 13 people who may get the 60 mcg dose of FluMos-v1 vaccine with adjuvant if this group is needed for the trial.

In **Part C**, we may enroll up to 26 people if needed to further study immune responses in this trial. This includes about 13 people who may get FluMos-v1 vaccine and 13 people who may get FluMos-v1 vaccine with adjuvant. We will decide what dose to give based on what we learn in Part B.

## WHAT ARE THE RISKS AND DISCOMFORTS OF BEING IN THE STUDY?

### Possible Risks of the FluMos-v1 Vaccine

The FluMos-v1 vaccine was given to people in this study in Part A. Based on risks of this vaccine seen in Part A and vaccines in general, you may experience any of the following: arm discomfort, redness of the skin or mild bruising at the injection site. Also, fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired and/or unwell. A few people who received the FluMos-v1 vaccine in Part A also had lymph node swelling and low neutrophil counts. FluMos-v1 may also have unknown risks. It has been tested in mice and rabbits, and the vaccine did not cause any unusual side effects and met the safety criteria to be tested in humans.

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

### Possible Risks of the Flucelvax® Vaccine

People experienced any of the following: arm discomfort, redness of the skin or mild bruising at the injection site. Also, fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired and/or unwell. These types of reactions are usually most common within the first 24 hours after vaccination and typically last 1 to 3 days. Over-the-counter medicine, like acetaminophen (Tylenol) or ibuprofen, may be used to help these symptoms.

Very rarely, a serious allergic reaction called anaphylaxis as described above may occur.

### Possible Risk of FluMos-v1 and Adjuvant

The FluMos-v1 vaccine has not been given with Adjuplex to people before. It may have unknown risks. It has been tested in animals. The vaccine did not cause any unusual side effects and met the safety criteria to be tested in humans. You may experience similar side effects to those for the FluMos-v1 vaccine without adjuvant as listed above.

**Possible Risks of Injections**

Temporary stinging, pain, redness, soreness, itchiness, swelling, or bruising may occur at the site of the injection.

**Possible Risks of Blood Drawing**

Blood drawing may cause pain, bruising, and lightheadedness or fainting. Rarely, an infection at the site where the blood is taken may happen.

**Possible Risks of Mucosal Sample Collection**

Samples collected by rubbing swabs over mucosal surfaces in the mouth, nose, or throat can cause brief discomfort or a little bleeding.

**Possible Risks of Apheresis**

Apheresis is generally safe, and side effects are rare. Pain, bruising or discomfort at the needle placement site may occur. Sometimes apheresis causes a tingling sensation around the lips, nose and mouth, coolness all over, and/or slight nausea. This can usually be relieved by slowing or temporarily stopping the apheresis or taking an antacid with calcium pill, like 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 and result in the loss of about one unit (one pint) of blood.

There are theoretical risks from re-infusion of the blood after processing by the machine such as infection or an adverse reaction to the blood components. However, this has not been seen in many thousands of volunteers who have undergone this or similar procedures to date. There may be other risks of apheresis that are unknown at this time.

During the leukapheresis procedure, your platelet count may decrease because platelets are collected with the white blood cells. Platelets are cells that help your blood to clot. Taking aspirin in combination with a lowered platelet count may increase your chance of developing bleeding. Therefore, you should not take aspirin or aspirin-containing drugs for 2 weeks after the procedure without physician approval.

**Unknown Safety Risks**

There may be side effects from the study vaccines - even serious or life-threatening ones- that we do not yet know about. Please tell the study staff about any side effect you think you are having. This is important for your safety.

**Possible Risks from Stored Samples**

We will collect blood samples from you during the study. We will keep these samples indefinitely for future research to learn more about flu virus, vaccines, the immune system, and other research questions. Results from research with your samples will not be in your medical record or reported to you.

Labeling of Stored Samples: Your stored samples will be labeled by a special code or number and not your personal information. Only the study team can link this code to you. Any identifying information about you (like name or date of birth) will be kept as confidential as allowable by law.

Risks of Stored Samples: There is a small chance that information from your medical records could be given to someone who should not get it without your permission. It is possible for someone to use that information to discriminate against you when you apply for insurance or employment. Similar problems may occur if you give information about yourself or agree to have your medical records released.

**Possible Risks of Data Sharing**

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

**Possible Other Risks**

We do not know if the study vaccine will change how your body responds to flu virus infections in the future.

You may not donate blood at a blood bank while taking part in this study. You may not donate blood for one year after the last experimental vaccine injection.

**What are the Risks Related to Pregnancy?**

We do not know how the experimental vaccines may affect a fetus or nursing infant. Therefore, women who can become pregnant must have a negative pregnancy test before the vaccine injection and agree to use effective birth control beginning at least 21 days before the injection until the end of the study. We will discuss effective methods of birth control with you.

Any time during the study you must tell the clinic staff right away if you become pregnant, your birth control method fails, or you think that you might be pregnant. If you are pregnant, you will be asked to continue with follow-up visits so that we can check your health. We will ask you the outcome of the pregnancy.

## WHAT ARE THE BENEFITS OF BEING IN THE STUDY?

You will not benefit from being in this study.

**Are there any potential benefits to others that might result from the study?**

The study is not designed to protect you from flu. We do not know if the vaccine will work. You and others may benefit in the future from the information that will be learned from the study. The study visits are used to check your health for research purposes, not to provide health care. However, we will tell you right away if any of your test results show a possible health problem.

## WHAT OTHER OPTIONS ARE THERE FOR YOU?

Before you decide if you want to be in this study, we will discuss other options that are available to you. You could choose not to take part in this study. You may be eligible for other VRC studies.

## DISCUSSION OF FINDINGS

### New information about the study

If we find out any new information that may affect your choice to take part in this study, we will get in touch with you to explain what we have learned. This may be information we have learned while doing this study here at the NIH or information we have learned from other scientists doing similar research in other places.

### Return of research results

At each visit you will be checked for any health changes or problems. Blood will be drawn at almost every study visit to check on your health. You will be told right away, in person, by phone call, or by text message if any of your test results show a health problem.

The results of this study may be reported in medical journals, on the internet or at scientific meetings. We will give you information about how to find the study results once they are available.

## EARLY WITHDRAWAL FROM THE STUDY

You may be removed from the research study by the researcher for any of the following reasons:

- You don't keep appointments or follow study procedures;
- You get a serious illness that needs ongoing medical care;
- You have a serious side effect thought to be due to the study vaccines;
- You become pregnant;
- You need to get treatment with a medication that affects your immune system (such as a steroid like prednisone);
- The study is stopped or cancelled;
- The researcher believes that it is in your best interest to remove you from the study;
- The study is stopped by regulatory agencies, the study sponsor, or study investigators. If this happens, we will tell you why.

If you agree to take part in this study, it is important for you to keep all of your appointments. Your participation in this study is completely voluntary. You can choose to stop taking part in the study at any time. There is no penalty or loss of benefits if you choose to leave the study. If you get the product administration during the study, we encourage you to take part in safety follow-up. It is important that we continue to check your health.

## STORAGE, SHARING AND FUTURE RESEARCH USING YOUR SPECIMENS AND DATA

### Will your specimens or data be saved for use in other research studies?

As part of this study, we are obtaining specimens and data from you. We will remove all the personal identifiers, such as your name, date of birth, address, or medical record number and label your specimens and data with a code so that you cannot easily be identified. The code will be linked through a key to information that can identify you. We plan to store and use these specimens and data for studies other than the ones described in this consent form that are going on right now, as well as studies that may be conducted in the future. These studies may provide additional information that will be helpful in understanding influenza, or other diseases or conditions. This could include studies to develop other research tests, treatments, drugs, or devices, that may lead to the development of a commercial product by the NIH and/or its research or commercial partners. There are no plans to provide financial compensation to you if this happens. Also, it is unlikely that we will learn anything from these studies that may directly benefit you.

If you agree to take part in this study, you give permission for your coded specimens and data to be stored and used for future research as described above.

### Will your specimens or data be shared for use in other research studies?

We may share your coded specimens and data with other researchers. If we do, we will not share it, so the code key, so the other researchers will not be able to identify you. They may be doing research in areas that are similar to this study or in other unrelated areas. These researchers may be at NIH, other research centers and institutions, or commercial entities.

If you agree to take part in this study, you give permission for your coded specimens and data to be stored and used for future research as described above.

If you change your mind and do not want us to store and use your specimens and data for future research, you should contact the research team member identified at the top of this document. We will do our best to comply with your request but cannot guarantee that we will always be able to destroy your specimens and data. For example, if some research with your specimens and data has already been completed, the information from that research may still be used. Also, for example, if the specimens and data have been shared already with other researchers, it might not be possible to withdraw them.

In addition to the planned use and sharing described above, we might remove all identifiers and codes from your specimens and data and use or share them with other researchers for future research at the NIH or other places. When we or the other researchers access your anonymized data, there will be no way to link the specimens or data back to you. We will not contact you to ask your permission or otherwise inform you before we do this. If we do this, we would not be able to remove your specimens or data to prevent their use in future research studies, even if you asked, because we will not be able to tell which are your specimens or data.

NIH policies require that your clinical and other study data be placed in an internal NIH database that is accessible to other NIH researchers for future research. Usually, these researchers will not

have access to any of your identifiers, such as your name, date of birth, address, or medical record number; and your data will be labeled with only a code. We cannot offer you a choice of whether your data to be placed in this database or not. If you do not wish to have your data placed in this database, you should not enroll in this study.

If you decide not to take part in this study, you may still take part in other studies at NIH.

**How Long Will Your Specimens and Data be Stored by the NIH?**

Your specimens and data may be stored by the NIH indefinitely.

**Risks of Storage and Sharing of Specimens and Data**

When we store your specimens and data, we take precautions to protect your information from others that should not have access to it. When we share your specimens and data, we will do everything we can to protect your identity, for example, when appropriate, we remove information that can identify you. Even with the safeguards we put in place, we cannot guarantee that your identity will never become known, or that no one will gain unauthorized access to your information. New methods may be created in the future that could make it possible to re-identify your specimens and data.

## PAYMENT

**Will you receive any type of payment for taking part in this study?**

Some NIH Clinical Center studies offer compensation for participation in research. The amount of compensation, if any, is guided by NIH policies and guidelines.

You will be compensated for your time and inconvenience by the NIH Clinical Research Volunteer Program. It is possible that you may have some expenses that are not covered by the compensation provided.

For all Groups the compensation is:

- $315 for the vaccination visit
- $25 total for the timely completion of all 7 days of an electronic diary
- $200 for each scheduled follow-up visit that includes a research blood draw
- $55 additional if a visit includes only a nose or throat swab
- $85 for all other clinic visits that do not include research blood draws or mucosal sample collection.
- $285 for optional apheresis

The total compensation for completion of all study visits is about $1,940 without apheresis and $2,025 with apheresis.

The total compensation you get is based on the number and type of study visits you complete. You will get the compensation about 2 weeks after each completed visit by direct deposit into a bank account that you specify to the Volunteer Payment Office.

If you are unable to finish the study, you will receive compensation only for visits you completed.

The study team will need your social security number in order to compensate you. If you don't provide your social security numbers and can still take part in the research study, however you may not be able to receive compensation.

With few exceptions, study compensation is considered taxable income that is reportable to the Internal Revenue Service (IRS). A "Form 1099-Other Income" will be sent to you if your total payments for research participation are $600 or more in a calendar year. If you have unpaid debt to the federal government, please be aware that some or all of your compensation may be automatically reduced to repay that debt on your behalf.

## REIMBURSEMENT

**Will you receive reimbursement or direct payment by NIH as part of your participation?**

Some NIH Clinical Center studies offer reimbursement or payment for travel, lodging or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

This study does not offer reimbursement for parents and participants, or payment of, hotel, travel, or meals.

## COSTS

**Will taking part in this research study cost you anything?**

NIH does not bill health insurance companies or participants for any research or related clinical care that you receive at the NIH Clinical Center.

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

## CONFLICT OF INTEREST (COI)

The NIH reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a COI Guide. You may ask your research team for a copy of the COI Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines or the guidelines of their home institution, but they do not need to report their personal finances to the NIH.

The NIH and the research team for this study have developed the FluMos-v1 vaccine being tested in this study. This means it is possible that the results of this study could lead to payments to NIH. By law, the government is required to share such payments with the employee inventors. You will not receive any money from the development of the vaccine.

## CLINICAL TRIAL REGISTRATION and RESULTS REPORTING

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

## CONFIDENTIALITY PROTECTIONS PROVIDED IN THIS STUDY

### Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The NIH and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Institutes of Health Intramural Institutional Review Board
- The study Sponsor, VRC, or their agent(s)

The researchers conducting this study and the NIH follow applicable laws and policies to keep your identifying information private to the extent possible. However, there is always a chance that, despite our best efforts, your identity and/or information about your participation in this research may be inadvertently released or improperly accessed by unauthorized persons.

In most cases, the NIH will not release any identifiable information collected about you without your written permission. However, your information may be shared as described in the section of this document on sharing of specimens and data, and as further outlined in the following sections.

Further, the information collected for this study is protected by NIH under a Certificate of Confidentiality and the Privacy Act.

### Certificate of Confidentiality

To help us protect your privacy, the NIH Intramural Program has received a Certificate of Confidentiality (Certificate). With this certificate, researchers may not release or use data or information about you except in certain circumstances.

NIH researchers must not share information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if requested by a court.

The Certificate does not protect your information when it:

- is disclosed to people connected with the research, for example, information may be used for auditing or program evaluation internally by the NIH; or
- is required to be disclosed by Federal, State, or local laws, for example, when information must be disclosed to meet the legal requirements of the federal Food and Drug Administration (FDA);
- is for other research;
- is disclosed with your consent.

The Certificate does not prevent you from voluntarily releasing information about yourself or your involvement in this research.

The Certificate will not be used to prevent disclosure to state or local authorities of harm to self or others including, for example, child abuse and neglect, and by signing below you consent to those

disclosures. Other permissions for release may be made by signing NIH forms, such as the Notice and Acknowledgement of Information Practices consent.

**Privacy Act**

The Federal Privacy Act generally protects the confidentiality of your NIH medical information that we collect under the authority of the Public Health Service Act. In some cases, the Privacy Act protections differ from the Certificate of Confidentiality. For example, sometimes the Privacy Act allows release of information from your record without your permission, for example, if it is requested by Congress. Information may also be released for certain research purposes with due consideration and protection, to those engaged by the agency for research purposes, to certain federal and state agencies, for HIV partner notification, for infectious disease or abuse or neglect reporting, to tumor registries, for quality assessment and medical audits, or when the NIH is involved in a lawsuit. However, NIH will only release information from your medical record if it is permitted by both the Certificate of Confidentiality and the Privacy Act.

**POLICY REGARDING RESEARCH-RELATED INJURIES**

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

**Problems or Questions**

If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator Maxwell Norris, MD; [redacted] Other researchers you may call are: Floreliz Mendoza, RN; [redacted] You may also call the NIH Clinical Center Patient Representative at [redacted] or the NIH Office of IRB Operations at [redacted] if you have a research-related complaint or concern.

**Consent Document**

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

**Adult Research Participant:** I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I consent to participate in this study.

| Signature of Research Participant | Print Name of Research Participant | Date |
|---|---|---|

**Investigator:**

| Signature of Investigator | Print Name of Investigator | Date |
|---|---|---|

**Witness to the oral short-form consent process only:**

**Witness:**

| Signature of Witness* | Print Name of Witness | Date |
|---|---|---|

***NIH ADMINISTRATIVE SECTION TO BE COMPLETED REGARDING THE USE OF AN INTERPRETER:**

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent and served as a witness. The investigator obtaining consent may not also serve as the witness.

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent but did not serve as a witness. The name or ID code of the person providing interpretive support is: _____.