A Phase 1 Open-Label, Comparator-Controlled, Dose-Escalation Study to Evaluate the Safety and Immunogenicity of a Single Dose of VRC H1ssF_3928 mRNA-LNP in Healthy Adults

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STATEMENT OF COMPLIANCE

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally-funded research. The Institutional Review Board/Independent Ethics Committee (IRB/IEC) must be registered with OHRP as applicable to the research.

The study will be carried out in accordance with the following as applicable:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11 (Electronic Records; Electronic Signatures), and 21 CFR Part 312 (Investigational New Drug Application), and/or 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice, and the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and Division of Microbiology and Infectious Diseases (DMID)
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

The signature below provides the necessary assurance that this study will be conducted according to all stipulations of the protocol including statements regarding confidentiality, and according to local legal and regulatory requirements, US federal regulations, and ICH E6(R2) Good Clinical Practice (GCP) guidelines.

Site Investigator Signature:

Signed: _____ Date: _____

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1. PROTOCOL SUMMARY

1.1 Synopsis

Rationale for Proposed Clinical Study

Influenza has been recognized as a global health threat since the 1918 influenza pandemic that caused an estimated 50 million deaths globally. Over 100 years of research on influenza has resulted in the availability of preventative vaccines, but seasonal vaccines remain only partially effective. The annual economic impact of seasonal influenza in the US is an estimated > $10 billion in direct medical expenses and > $85 billion in total economic impact[1].

Influenza disease is caused by an orthomyxovirus that has a wide host range including birds, swine, and humans. Animal reservoirs periodically permit the introduction of new viral strains into the human population with varying degrees of transmission from person-to-person. Animal reservoirs are thought to be the source of new pandemic strains due to influenza's segmented, negative sense ribonucleic acid (RNA) genome that can undergo reassortment during coinfection in animal hosts. Introduction of a reassorted virus to a susceptible population, called antigenic shift, can lead to worldwide outbreaks such as the 2009 H1N1 pandemic. Moreover, influenza's RNA genome is replicated by a virus-encoded and error-prone RNA polymerase that provides the mechanism for antigenic drift, defined as small but significant changes to immunogenic epitopes on hemagglutinin (HA), neuraminidase (NA), and other viral proteins. Seasonal changes due to antigenic drift can replace previously circulating strains by conferring immune escape that occurs at both individual and population levels. These changes cause vaccine efficacy to change over time, requiring ongoing surveillance of circulating strains to develop new seasonal influenza vaccines. Twice yearly, the World Health Organization (WHO) recommends which strains to include in the seasonal vaccine, and the WHO established the Global Influenza Surveillance and Response System in 1952 to monitor the spread of antigenically distinct viral strains. This surveillance team, consisting of over 148 National Influenza Centers in 123 countries, tested an average of 3.4 million specimens annually between 2014-2019 and shares 20,000 virus samples each year to WHO collaborating centers. Despite this investment, influenza vaccine protection is suboptimal, and development of vaccines that overcome these problems could improve vaccine coverage.

In addition, the infrastructure for current vaccine manufacture largely relies on virus propagation in embryonated chicken eggs which subjects the supply chain to multiple vulnerabilities including the availability of eggs, time needed for establishment of viral stocks and production, risks of exposure to chicken proteins co-purifying with vaccines, the need for virus inactivation using potentially carcinogenic compounds such as formaldehyde, and the introduction of egg adaptations during virus propagation that can affect vaccine response. Methods for vaccine production that are not susceptible to these problems may enable more rapid response in the face of a new pandemic strain, as has been demonstrated by the rapid development of vaccine candidates for SARS-CoV-2.

The vaccine product being evaluated in this study is a modified messenger ribonucleic acid (mRNA) encoding a headless HA stabilized stem construct, derived from influenza A/New Caledonia/20/1999 (H1N1) and covalently linked to *Helicobacter pylori (H. pylori)* ferritin. The mRNA is encapsulated in a lipid nanoparticle (LNP) for delivery. This formulation is being tested as a proof-of-concept for a universal influenza vaccine approach, leveraging an

immunogen that is designed to elicit universally protective responses. The mRNA in this vaccine encodes for a protein immunogen that has already been tested in a human clinical trial conducted by the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center (VRC) (VRC 321, NCT03814720) [2]. The protein immunogen has been shown to have an acceptable safety profile as described below.

NCT03814720 was an open label dose and age-escalation study looking at the safety, tolerability, and immunogenicity of receiving up to two doses. There were no reported serious adverse events (SAEs), severe, life-threatening or fatal adverse events (AEs).

For the vaccine product to be evaluated in the current study, H1ssF_3928 mRNA Vaccine, modified mRNA is encapsulated in LNP consisting of 4 lipid components: an ionizable lipid, a neutral structural lipid, cholesterol, and a diffusible polyethylene glycol (PEG) lipid conjugate. Testing of this mRNA-LNP influenza vaccine in mouse models has demonstrated its ability to elicit an antibody response to both homologous and heterosubtypic influenza viruses. Several mRNA-LNP seasonal influenza vaccines are currently in clinical development, and there have been clinical trials of mRNA seasonal influenza vaccines and potential pandemic influenza vaccines (H7N9 and H10N8). In addition, two mRNA-LNP vaccines against SARS-CoV-2 have been approved by the FDA.

Study Design

This is a Phase 1 open-label, comparator-controlled, dose escalation study of an intramuscularly (IM) administered mRNA-LNP vaccine encoding for VRC H1ssF_3928, including up to 50 healthy adult volunteers aged 18 to 49 years, designed to assess vaccine safety and immunogenicity at varying doses. The vaccine drug product name is "H1ssF_3928 mRNA Vaccine." Eligible participants will be sequentially enrolled into dosing groups (10 mcg, 25 mcg, 50 mcg, and selected optimal dose) of the VRC H1ssF_3928 mRNA-LNP vaccine. Dosing will commence at the lowest dose (10 mcg) and only escalate to the next highest dose if safety concerns are not identified. Up to 10 subjects will be enrolled per dosing group. Ten separate participants will be enrolled to receive standard quadrivalent inactivated influenza vaccine (IIV4). Subjects receiving IIV4 will be followed for safety but only their immune responses will be compared to those of participants receiving VRC H1ssF_3928 mRNA-LNP vaccine.

For each VRC H1ssF_3928 mRNA-LNP dosing group, the first two subjects enrolled will be considered the sentinel subgroup **Table 1**. After the two subjects in the Low Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a review of the reactogenicity and safety data collected through Day 3 for both subjects. This review will be conducted by a safety review committee (SRC), as indicated and detailed in Section 10.1.6. Approval by the reviewing group will allow continued enrollment of the remaining 8 Low Dose Group subjects (expanded subgroup) to resume to complete enrollment of 10 participants. After Low Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 for all Low Dose Group subjects. Approval by the SRC will allow dose escalation and initiation of enrollment of the Medium Dose Group sentinel subgroup.

After the two subjects in the Medium Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the Low Dose Group. Approval will allow continued enrollment of Medium Dose Group subjects (expanded subgroup) to complete enrollment of 10 participants. After Medium

Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and adverse event information through Day 7 and clinical laboratory results through Day 8 for all Medium Dose Group subjects. Approval by the SRC will allow dose escalation and initiation of enrollment of the High Dose Group sentinel subgroup.

After the two subjects in the High Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the Low and Medium Dose Groups. Approval will allow continued enrollment of High Dose Group subjects (expanded subgroup) to complete enrollment of 10 participants. After High Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and adverse event information through Day 7 visit and clinical laboratory results through Day 8 for all High Dose Group subjects.

Reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 from the first three dosing groups will guide the SRC during their review meeting to the selection of an optimal dose group to include an additional 10 subjects who will receive the optimal dose of mRNA-LNP (see **Section 4.2**). Vaccinations will be performed by licensed vaccine administrators while assessments will be performed by the clinical team.

All subjects will complete a subject memory aid for 14 days after study vaccination. Unsolicited, non-serious AEs will be collected through 28 days after the vaccination. MAAEs, NOCMCs, AESIs corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, SAEs, and ILIs will be collected from the time of vaccination throughout the study period. AESIs corresponding to myocarditis, pericarditis, or myopericarditis will be collected from the time of vaccination through Day 57. Subjects' participation will be approximately 12 months. **Table 2** provides a detailed outline of the study schedule.

Blood samples and nasal specimens will be obtained at specified time points following vaccination for safety laboratory assessments and assessments of innate and adaptive immune responses.

Table 1: Study Schema

Group	**Subgroup**	**VRC H1ssF_3928 mRNA LNP Dose[1]**	**Number of Subjects**
Low Dose	Sentinel[2]	10 mcg	2
	Expanded[3]	10 mcg	8
Medium Dose	Sentinel[2]	25 mcg	2
	Expanded[3]	25 mcg	8
High Dose	Sentinel[2]	50 mcg	2
	Expanded[3]	50 mcg	8

Group	Subgroup	VRC H1ssF_3928 mRNA LNP Dose[1]	Number of Subjects
Optimal Dose	Complete	Selected mcg	10
	Subgroup	**IIV4 Dose[4]**	
IIV4	Immunogenicity Comparator	60 mcg (15 mcg / antigen)	10
Total			50

[1] VRC H1ssF_3928 mRNA-LNP will be given as an IM injection at Day 1.

[2] SRC review of the reactogenicity and AE data available from enrollment through Day 3 for the first 2 sentinel subjects (VRC H1ssF_3928 mRNA- LNP) will be conducted prior to initiation of enrollment of the remaining subjects in the Group. If deemed necessary by the SRC, the Safety Monitoring Committee (SMC) will review the safety data.

[3] SRC review of the reactogenicity and AE data available from enrollment through the post vaccination Day 7 visit and clinical laboratory results through Day 8 for the Group will be conducted prior to initiation of enrollment in the next highest dosing group. If deemed necessary by the SRC, the SMC will review the safety data.

[4] Licensed quadrivalent inactivated influenza vaccine (IIV4) will be given as an IM injection at Day 1.

Study Objectives

- **Primary**
  - To assess the safety of a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults (18-49 yrs.) at doses of 10 mcg, 25 mcg, and 50 mcg.
- **Secondary**
  - To assess serum antibody responses to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.
- **Exploratory**
  - To assess the anti-ferritin antibody response (to human and *H. pylori* ferritin) to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg.
  - To assess the durability of the immune response to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.
  - To assess antibody breadth to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.

- To assess the B cell and/or T cell, including T follicular helper cell, responses to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.

Study Endpoints

- **Primary**
  - Number and percentage of participants experiencing solicited local and systemic reactogenicity AEs through 14 days
  - Number and percentage of participants experiencing any unsolicited AEs through 28 days
  - Number and percentage of participants experiencing any medically-attended adverse events (MAAEs), new-onset chronic medical conditions (NOCMCs), adverse events of special interest (AESIs) corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, influenza-like illnesses (ILIs), and SAEs through one year (Day 366) and AESIs corresponding to myocarditis, pericarditis, or myopericarditis through 57 days (Day 57)
  - Number and percentage of participants experiencing clinical laboratory AEs through 57 days (Day 57)
- **Secondary**
  - Geometric mean titer (GMT) of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - Geometric mean fold rise (GMFR) from baseline (Day 1) of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - GMT of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - GMFR from baseline (Day 1) of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - GMT of anti-stalk serum antibodies, measured by enzyme linked immunosorbent assay (ELISA), at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group

- GMFR from baseline (Day 1) of anti-stalk serum antibodies, measured by ELISA, at Days 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group

- **Exploratory**
  - Measures of anti-ferritin antibody response (to human and *H. pylori* ferritin) to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg at Days 1, 29, and 181 by analysis group and stratified by prior season's receipt and/or subsequent receipt (where applicable) of seasonal influenza vaccine within each analysis group
  - GMT of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - GMT of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt and/or subsequent receipt of seasonal influenza vaccine within each analysis group
  - GMT of anti-stalk serum antibodies, measured by ELISA, at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt and/or subsequent receipt of seasonal influenza vaccine within each analysis group
  - Neutralizing antibody response to divergent H1 influenza virus isolates and other group 1 non-H1 influenza virus isolates at Day 1 and Day 29 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - Anti-stalk serum antibody response to divergent H1 influenza virus isolates and other group 1 non-H1 influenza virus isolates at Day 1 and Day 29 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
  - Exploratory immunogenicity evaluations may include B cell assays, T cell assays, and T follicular helper cell assays at selected time points in the entire study population or subsets of participants by analysis group and stratified by prior season's receipt and/or subsequent receipt (where applicable) of seasonal influenza vaccine within each analysis group

Eligibility

A potential participant is eligible to join this trial if he/she meets all the following inclusion criteria and none of the following exclusion criteria:

Inclusion criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provide written informed consent prior to initiation of any study procedure.

2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Are males or non-pregnant, non-breastfeeding females, 18 to 49 years of age, inclusive at time of screening and enrollment.
4. Must agree to collection of venous blood and nasal absorption specimens per protocol and enrollment in DMID Protocol No. 19-0025 biorepository protocol for secondary research and use of residual biologic specimens.
5. Are in good health[1] and do not have clinically significant medical, psychiatric, chronic or intermittent health conditions including those listed in Exclusion Criteria (Section 1).

   [1] *As determined by medical history, medication use, and physical examination to evaluate ongoing chronic medical or psychiatric diagnoses or conditions, defined as those that have been present for at least 90 days, which would not affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. These medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, emergency room [ER] or urgent care for condition [excluding musculoskeletal conditions], or invasive medical procedure and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose or in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening or treatment of continued symptoms of medical diagnosis or condition. Note: Low dose topical corticosteroids as outlined in the Subject Exclusion Criteria (see Section 1) as well as herbals, vitamins and supplements are permitted.*
6. Does not have an ongoing symptomatic condition[2] for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan.

   [2] *e.g., ongoing fatigue without a diagnosis for symptom.*
7. Pulse is 50 to 100 beats per minute, inclusive.
8. Systolic blood pressure is 90 to 140 mmHg, inclusive.
9. Diastolic blood pressure is 55 to 90 mmHg, inclusive.
10. Body mass index (BMI) of 18 kilograms/square meter (kg/m^2) (inclusive) to <35 kg/m^2 at screening
11. Women of childbearing potential[3] must agree to use or have practiced true abstinence[4] or use at least 1 acceptable primary form of contraception.[5,6]

    [3] *Not of childbearing potential – post-menopausal females (defined as having a history of amenorrhea for at least one year) or a documented status as being surgically sterile (hysterectomy, bilateral oophorectomy, salpingectomy, or Essure® placement with history of documented radiological confirmation test at least 90 days after the procedure).*

[4] True abstinence is 100% of time no sexual intercourse (male's penis enters the female's vagina). (Periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception).

[5] Acceptable forms of primary contraception include monogamous relationship with a vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the study product, tubal ligation, intrauterine devices, birth control pills, and injectable/implantable/insertable hormonal birth control products.

[6] Must use at least one acceptable primary form of contraception or true abstinence for at least 30 days prior to receipt of study product and at least one acceptable primary form of contraception or true abstinence for at least 30 days following receipt of study product.

12. Women of childbearing potential[3] must have a negative serum HCG pregnancy test at screening and a negative urine HCG pregnancy test within 24 hours prior to the study vaccination.
13. Male participants receiving VRC H1ssF_3928 mRNA-LNP must agree to refrain from donating sperm and to use contraception until Day 60 after vaccination.[7, 8]

[7] Acceptable contraception includes abstinence from intercourse with a female of childbearing potential or use of a male condom when engaging in any activity that allows for passage of ejaculate to a female during the intervention period for at least 60 days after study vaccination.

[8] Males in the immunogenicity comparator group do not have to refrain from sperm donation or abstain from intercourse or agree to use a male condom for purposes of this study.

14. Must have received at least one licensed seasonal influenza vaccine within the previous 5 seasons.

Exclusion criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Have an acute illness[9] or fever (body temperature ≥38.0°C/100.4°F), as determined by the site Principal Investigator (PI) or appropriate sub-investigator, within 72 hours prior to study vaccination.

[9] An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.

2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation.[10]

[10] Including acute, subacute, intermittent or chronic medical disease or condition that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this trial.

3. Has a screening laboratory[11] >Grade 1.

[11] White blood cell count, absolute neutrophil count, absolute lymphocyte count, hemoglobin, platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT),

fibrinogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, lipase

4. Has a positive urine toxicology screen (i.e., non-prescribed amphetamines, cocaine, and opiates).
5. ECG is deemed to be clinically significant by the PI or appropriate sub-investigator.
6. Total iron binding capacity, iron, ferritin and troponin (hsTnI) outside the laboratory normal range at screening.
7. Have any known or suspected immunosuppressive condition, acquired or congenital, or autoimmune conditions as determined by history and/or physical examination.
8. Have immunosuppression as result of treatment including a recent history (within 6 months prior to administration of study vaccine) or use of immunosuppressive or other immune-modifying drugs.
9. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
10. Have known active or recently active (12 months) neoplastic disease or a history of any hematologic malignancy. Non-melanoma, treated, skin cancers are permitted.
11. Have known human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection at screening.
12. Have a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or HIV types 1 or 2 antibodies at screening.
13. Have known chronic liver disease, including fatty liver disease.
14. Have a known allergy or severe allergic reaction to any components of the study vaccine (including polyethylene glycol [PEG]) or the seasonal influenza vaccine (including egg protein).
15. Have a history of a severe reaction following previous immunization with an investigational, authorized, or approved influenza vaccine or mRNA or LNP-containing vaccine.
16. Have a history of Guillain-Barré Syndrome.
17. Have a known history of myocarditis or pericarditis.
18. Have a history of alcohol or drug abuse within 3 years prior to study vaccination.
19. Have any diagnosis, current or past, of schizophrenia, bipolar disease or other psychiatric diagnosis that may interfere [12] with subject compliance or safety evaluations.

    [12] *As determined by the site PI or appropriate sub-investigator.*
20. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination.
21. Have taken oral or parenteral (including intra-articular) corticosteroids of any dose within 30 days prior to study vaccination. Intranasal or topical (skin or eyes) corticosteroids are permitted.

22. Have taken high-dose inhaled or nebulized corticosteroids[13] within 30 days prior to study vaccination.

    [13] *High-dose defined as per age as using inhaled high-dose per reference chart in the National Heart, Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma (EPR-3) or other lists published in UPTODATE.*

23. Have any significant disorder of coagulation requiring ongoing or intermittent treatment.
24. Have received seasonal influenza vaccine within 90 days prior to enrollment or plans to receive a seasonal influenza vaccine 60 days after study vaccination.
25. Have received any approved or authorized vaccines other than seasonal influenza vaccine within 60 days before enrollment or plans to receive an approved or authorized vaccine 60 days after study vaccination.
26. Have a known history of documented influenza infection within the past 90 days.
27. Have a history of receipt of an investigational H1 influenza vaccine within the past 10 years.
28. Have a history of receipt of a ferritin-based vaccine or an investigational H5 influenza vaccine.
29. Previous participation in DMID Protocol No. 18-0010 human influenza challenge study.
30. Received immunoglobulin and/or any blood products (except Rho D immunoglobulin) within the 90 days prior to study vaccination.
31. Received an experimental agent[14] within 60 days prior to the study vaccination or expect to receive another experimental agent during the trial-reporting period.[15]

    [14] *Including vaccine, drug, biologic, device, blood product, or medication.*

    [15] *Other than from participation in this trial*

32. Are participating or plan to participate in another clinical trial with an interventional agent[16] that will be received during the trial-reporting period.[17]

    [16] *Including licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication.*

    [17] *Approximately 12 months after the first study vaccination.*

33. The subject has any abnormality or permanent body art (e.g., tattoo) that, in the opinion of the investigator or appropriate sub-investigator, would obstruct the ability to observe local reactions at the injection site.
34. Donation of blood or blood products within 30 days prior to dosing and plans to donate within 60 days following dosing.
35. Has had significant exposure to someone with laboratory-confirmed SARS-CoV-2 infection or influenza in the 14 days prior to screening or during the period between screening and enrollment visit.[18]

    [18] *Defined by the Centers for Disease Control and Prevention (CDC) as a close contact with someone who has COVID-19.*

36. Has had a positive SARS-CoV-2 test (home or laboratory-based) within 14 days prior to the screening visit or during the period between screening and enrollment visits.

Study Phase

Phase 1

Study Population

50 healthy adults 18 to 49 years of age inclusive.

Sites

One US site.

Study intervention:

Investigational Vaccine: H1ssF_3928 mRNA Vaccine. Modified mRNA encoding the VRC H1ssF_3928 sequence which is a headless HA stabilized stem construct derived from influenza A/New Caledonia/20/1999 (H1N1) covalently linked to *H. pylori* ferritin. The mRNA is encapsulated in LNP for delivery. The product will be administered IM on Day 1 at a dose of 10 mcg, 25 mcg or 50 mcg. Comparison group will receive licensed IIV4 as an immunogenicity comparator product.

Study Duration: 26 months

Participant Duration: Up to 54 weeks

Safety

This protocol will utilize a safety monitoring committee (SMC). The SMC will have an organizational meeting and will conduct a review of safety data if a halting rule is met (Refer to Section 7.1.1), if the internal safety review committee (SRC) requests a review of the data, or ad hoc when requested by the sponsor, investigators or SMC. In addition, the SMC will review the safety, tolerability, and trial progress at the conclusion of the trial.

1.2 Schedule of Activities (SoA)

Table 2: Schedule of Activities

Procedures	Study Visit 0 Screening Day -14 to -1	Enrollment/Baseline Visit 1, Day 1	Study Visit 2 Day 3 +/- 1 day	Study Visit 3 Day 8 +/- 2 days	Study Visit 4 Day 15 +/- 2 days	Study Visit 5 Day 29 +/- 3 days	Study Visit 6 Day 57 +/- 3 days	Study Visit 7 Day 85 +/- 7 days	Study Visit 8 Day 181 +/- 7 days	Study Visit 9 Day 366 +/- 7 days	Unscheduled Visit[21]	Early Termination Visit
Type of Visit	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**
Informed consent[1]	X											
Review / Confirm Eligibility Criteria	X	X										
Abstinence and contraception counseling[16]	X	X	X	X	X	X	X					X

Procedures	Study Visit 0 Screening Day -14 to -1	Enrollment/Baseline Visit 1, Day 1	Study Visit 2 Day 3 +/- 1 day	Study Visit 3 Day 8 +/- 2 days	Study Visit 4 Day 15 +/- 2 days	Study Visit 5 Day 29 +/- 3 days	Study Visit 6 Day 57 +/- 3 days	Study Visit 7 Day 85 +/- 7 days	Study Visit 8 Day 181 +/- 7 days	Study Visit 9 Day 366 +/- 7 days	Unscheduled Visit[21]	Early Termination Visit
Demographics[2]	X											
Medical history[3]	X	X										X
Influenza vaccination history[3]	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication review[4]	X	X	X	X	X	X	X	X	X	X	X	X
Complete physical exam (including height and weight)[5]	X											
Targeted physical exam[5]		X	X	X	X	X	X	X	X	X	X	X
ECG (12-lead)	X		X	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]
Vital signs[6]	X	X	X	X	X	X	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]
Hematology[7]	X	X		X		X	X				X[17]	X[17]
Coagulation studies[8]	X	X		X		X	X				X[17]	X[17]
Serum chemistry[9]	X	X		X		X	X				X[17]	X[17]
Troponin	X	X[18]	X[18]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]	X[17]
Blood for HIV 1 and 2 antigen/antibody, HBV, and HCV	X											
Serum HCG	X											
Urine Toxicology Screen[10]	X											
Urine HCG[11]		X										
Administer study product		X										
Solicited AEs[12]		X	X	X	X							
Non-serious unsolicited AEs[13]		X	X	X	X	X						
MAAEs, NOCMCs, AESIs, SAEs[14]		X	X	X	X	X	X	X	X	X	X	X
Nasal absorptive matrix for secondary research		X[19]	X	X	X	X	X				X[17]	X[17]
Serum for immunologic assessments (e.g., MN, Stalk, Breadth)		X[19, 20]	X	X	X	X[20]	X	X	X[20]	X	X[17]	X[17]
PBMC and plasma collection for exploratory and secondary research		X[19]		X	X	X	X	X	X	X	X[17]	X[17]

Procedures	Study Visit 0 Screening Day -14 to -1	Enrollment/Baseline Visit 1, Day 1	Study Visit 2 Day 3 +/- 1 day	Study Visit 3 Day 8 +/- 2 days	Study Visit 4 Day 15 +/- 2 days	Study Visit 5 Day 29 +/- 3 days	Study Visit 6 Day 57 +/- 3 days	Study Visit 7 Day 85 +/- 7 days	Study Visit 8 Day 181 +/- 7 days	Study Visit 9 Day 366 +/- 7 days	Unscheduled Visit[21]	Early Termination Visit
Whole blood PAXgene tube for secondary research		X[19]	X	X							X[17]	X[17]
ILI Visit (e.g., nasopharyngeal swab for influenza virus RT-PCR testing)[15]											X	

1. Prior to any study related procedures, participants will be provided information about the study and informed consent will be obtained from the participant.
2. The investigator or appropriately delegated study staff will obtain demographic data.
3. The investigator or appropriately delegated study staff will obtain medical history, influenza vaccination history and information necessary to determine eligibility. Since screening may occur up to 14 days before enrollment, if eligibility and medical history are taken at a screening visit, they will be updated on the day of enrollment, before administration of study product. Demography will not be updated.
4. Medications taken by the participant in the 30 days before enrollment will be recorded (while all medications taken from 90 days prior to screening, whether on the same day as enrollment or not, will be solicited). At subsequent visits, until Visit 9 (Day 366) updates to previous concomitant medications and additional medications taken in the interim will be recorded.
5. Physical examination will occur for all participants at Visit 0. Participants may receive an optional targeted physical exam at any other clinic visits at investigator discretion.
6. Vital signs include oral temperature, heart rate, and blood pressure
7. Hematology includes white blood cell count with differential, hemoglobin and platelet count. If low levels of Hb are found, it will be under the PI's discretion to decide the amount of blood, if any, that can be withdrawn in the following visit
8. Coagulation studies includes prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen. If abnormal levels are found, it will be under the PI's discretion to continue with protocol procedures such as blood withdrawn at the following visit.
9. Serum chemistry includes creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, lipase, total iron binding capacity, serum iron, and ferritin
10. Negative drug urine toxicology result (amphetamines, cocaine, and opiates) required at screening.
11. Urine pregnancy test will be performed for females of childbearing potential within the 24 hours prior to study product administration. Result must be negative before study product can be administered.
12. Solicited AEs will first be collected 30 minutes after administration of vaccine (immediate reactogenicity). Additional solicited AEs will be collected by memory aid (eDiary) on Days 1-14 for all participants. Solicited AEs consist of immediate reactogenicity, local reactogenicity and systemic reactogenicity (constitutional signs and symptoms as defined in the protocol).
13. Non-serious unsolicited AEs will be collected and recorded from Day 1 after administration of vaccine for all participants until Day 28
14. MAAEs, NOCMCs, and SAEs as defined in the protocol, will be collected from Day 1, following vaccination, until the end of study follow-up (Day 366). AESIs corresponding to myocarditis, pericarditis, and myopericarditis will be collected from Day 1, following vaccination, until Day 57. AESIs, corresponding to any Grade 2 or greater decrease in lymphocyte or neutrophil count will be collected from Day 1, following vaccination, until the end of study follow up (Day 366).
15. Participants will be required to report influenza-like illness starting from enrollment through day 366. Participants will have the ability to notify the study team through a weekly electronic memory aid starting on Day 7. Participants experiencing an influenza-like illness will have an unscheduled visit and a

nasopharyngeal (NP) swab will be obtained for influenza virus testing by RT-PCR. ILI symptoms will be collected from the day of enrollment through Day 366.

16. Screening will include counseling women of childbearing potential and sexually active men on abstinence or approved contraceptive methods. If an early termination visit is performed before Day 30 for a woman of childbearing potential, or before Day 60 for a sexually active man, contraceptive counseling will be performed.
17. Evaluation performed at the discretion of the investigator
18. Blood sample for troponin measurements will be collected, stored, and tested at the discretion of the PI and will not be included in the participant's electronic medical record.
19. Sample collection will occur prior to vaccination.
20. Exploratory anti-ferritin antibody assays.
21. Unscheduled visits may be conducted by phone or IRB-approved telehealth means if in-person visits are not feasible. If an unscheduled visit is performed to evaluate, in the judgment of the site investigator, a case of suspected myocarditis and/or pericarditis, the site will coordinate an appropriate diagnostic workup to make a determination of probable or confirmed myocarditis and/or pericarditis. The suspected myocarditis and/or pericarditis case should be reported to the DMID Pharmacovigilance Group within 24 hours of site awareness.

2. INTRODUCTION

2.1 Study Rationale

Influenza is a contagious respiratory illness caused by influenza viruses. Illness ranges from mild to severe and in some cases result in death. Worldwide, it is estimated that between 291,000 and 646,000 influenza deaths occur per year [3]. In the United States, annual influenza vaccination is recommended for all persons over six months of age for the prevention of influenza caused by both influenza A subtype viruses and subtype B viruses included in the respective season's vaccine. In the United States the average effectiveness of influenza vaccine over the course of 18 seasons was between 10% to 60% [4]. The influenza virus' propensity for rapid antigenic drift resulting from changes to immunogenic epitopes on important viral proteins, including hemagglutinin (HA) and neuraminidase (NA) is likely partly responsible for variable vaccine effectiveness. In addition, antigenic shift due to genetic reassortments from animal influenza viruses, can lead to novel influenza strains to which the majority of the population has no pre-existing immunity and result in a major pandemic. Improved influenza vaccines that target conserved influenza viral epitopes and induce broader protection against antigenically drifted and shifted influenza viruses are needed. To address this unmet need, we are evaluating the safety and immunogenicity of H1ssF_3928 mRNA, a messenger ribonucleic acid (mRNA) encoding a headless HA stabilized stem construct, derived from influenza A/New Caledonia/20/1999 (H1N1) and covalently linked to *Helicobacter pylori* ferritin.

To elicit antibodies that would protect against multiple influenza viruses over multiple years, the Vaccine Research Center (VRC), as well as other groups, have engineered HA monomers in which HA1 and HA2 proteins have been modified to remove the variable head domain and to stabilize the remaining stem in a native-like tertiary and quaternary structure. [5-8] The VRC vaccine candidate, H1ssF_3928 Protein, has been designed, engineered, and confirmed to elicit a vigorous immune response against multiple influenza subtypes. Candidate vaccine H1ssF_3928 Protein is being evaluated in a dose-escalation, stratified age-group Phase 1 trial for safety and efficacy at the NIH Clinical Center (CC) (NCT03814720).

Nucleoside-modified mRNA safety, ease of production, and relatively low cost to manufacture, make modified mRNA-lipid nanoparticle (LNP) vaccines ideal to test in humans as an influenza vaccine approach. The purpose of the clinical trial proposed here is to test the safety and immunogenicity of the H1ssF_3928 antigen when delivered as an mRNA-LNP.

2.2 Background

2.2.1 Purpose of Study

This is a Phase 1, open-label, comparator-controlled dose escalating study of an intramuscularly (IM) administered mRNA-LNP vaccine, H1ssF_3928 mRNA Vaccine, encoding for VRC H1ssF_3928, including up to 50 healthy adult volunteers aged 18 to 49 years, designed to assess vaccine safety and immunogenicity at varying doses.

2.2.1.1 Influenza

Epidemic influenza is a highly infectious disease that occurs annually throughout the temperate regions in both hemispheres in their respective winter months [9]. Influenza infection occurs in all age groups and is a major public health threat, as it has the ability to spread rapidly through

populations and affect a large number of people. While infection rates are generally highest among school age children, the highest rates of serious morbidity and mortality occur among individuals over 65 years of age and in children less than 1 year of age. [10] Underlying medical conditions such as chronic debilitating disease, chronic cardiac, pulmonary, kidney and metabolic disorders, and various types of immunosuppression increase the likelihood of potential complications. [11]

Uncomplicated influenza illness is characterized by the abrupt onset of constitutional symptoms including fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis. Influenza virus infection can also lead to more serious illness such as primary viral pneumonia and exacerbation of underlying medical conditions. Common complications include bacterial pneumonia, sinusitis, and otitis media. The estimated annual number of influenza-associated hospitalizations in the United States is 226,000 and the estimated annual number of deaths is 34,470. [12, 13]

2.2.1.2 Candidate Vaccine - H1ssF_3928 mRNA Vaccine

The investigational vaccine drug product consists of modified nucleoside mRNA encapsulated in lipid nanoparticles (LNP), comprised of four lipid components: (an ionizable cationic lipid [Bis(2-butyloctyl) 10-(N-3-(pyrrolidin-1-yl)propyl)nonanamido)nonadecanedioate], 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC], non-animal derived cholesterol, and 2-(polyethylene glycol)-2000-N,N-ditetradecylacetamide [PEG lipid]). Upon immunization with mRNA-LNPs, endocytosis by host cells leads to LNP-mediated intracellular delivery of the mRNA. The mRNA encodes influenza H1 stabilized stem (H1ss) amino acid sequence covalently fused to *H. pylori* ferritin (F) amino acid sequence. The chimeric construct is expressed from genetically-fused, in-frame H1ss and F coding sequence (cds) as a single polypeptide. The N-terminal stabilized stem derives from A/New Caledonia/20/1999 (H1N1) [NC99] HA from which the residues comprising the head domain have been removed and the stem domain has been stabilized to maintain antigenicity and binding to selected monoclonal antibodies known to broadly neutralize group 1 influenza A viruses through binding a conserved HA stem epitope. [8] The ferritin amino acid sequence is that of bacterium *H. pylori*. Upon translation of the H1-ferritin fusion protein, the H1ssF_3928 protein is secreted from the host cells and generates an immune response.

The native HA protein, resident on the influenza virion surface or the engineered ferritin core surface, assembles as a homotrimer of three HA1 and HA2 polypeptide heterodimers. Each heterodimer tertiary structure contains a globular head domain and a highly helical stem domain. The H1ssF_3928 vaccine construct has been modified to remove the HA head domain, which is both immunodominant and variable from strain to strain and over time, properties that contribute substantially to the efficacy and durability problems characteristic of the annual influenza vaccine. Both HA-forming heterodimer polypeptides, HA1 and HA2 residues contribute to the stem HA domain, requiring iterative and creative structure-based design and experimental analysis to identify a correctly-folded, stable stem tertiary structure in the absence of head residues. [8] The fruit of that work is a stem domain vaccine, in this case derived from an H1 strain of influenza, that is stabilized in a native-like tertiary structure and presents to the recipient's immune system conserved but immunologically subdominant epitopes that are a hallmark of the stem domain. Expression from genetically-fused, in-frame, stabilized stem and ferritin cds has been confirmed to assemble to a ferritin 24-mer polyhedron with H1ss

homotrimers projecting from the eight axes of threefold symmetry and, in mouse and ferret nonclinical studies, to elicit protective immune responses. [8]

Non-clinical studies have been conducted in mice and ferrets with research grade H1ssF_3928 protein with the adjuvant monophosphoryl lipid A-synthetic trehalose dicorynomycolate, formerly also called Ribi adjuvant. [8] After three doses, in both species, the H1ssF_3928 protein elicited Group 1 HA binding and neutralizing antibody responses and protected against challenge with influenza A/Vietnam/2004 (H5N1) [VN04], whereas empty ferritin nanoparticles were not protective. These studies demonstrate rationale for evaluation of H1ssF_3928 immunogen in the clinical trial.

In addition to the above non-clinical studies of the H1ssF_3928 protein, this immunogen was also evaluated in animals when delivered as an mRNA-LNP. Balb/c mice were immunized with the H1ssF_3928 mRNA-LNP in three intradermal (ID) or IM immunizations of 3 mcg of mRNA at 4-week intervals. Blood was obtained at 4 weeks following the last injection for serological analysis by enzyme linked immunosorbent assay (ELISA) against either the homologous H1 NC99 or the heterosubtypic H5 VN04. H1ssF_3928 mRNA-LNP was immunogenic when administered by either route (see Investigator's Brochure).

In toxicity studies of the protein antigen known as H1ssF_3928, New Zealand White rabbits received IM injections of either 60 or 180 mcg/kg VRC-FLUNPF099-00-VP vaccine once every three weeks for a total of three doses. The H1ssF_3928 protein injections were well tolerated, supporting the safety of the H1ssF_3928 protein, the same protein expressed in the proposed mRNA-LNP vaccine formulation.

In a GLP repeat dose toxicity study, VRC H1ssF_3928 mRNA-LNP was administered via IM injection to Wistar Han rats on Days 1, 15, and 29, at a dose of 50 mcg/dose/day (with a total of three IM injections). There were local and systemic changes related to VRC H1ssF_3928 mRNA-LNP. All vaccine related effects were considered to reflect a normal immune/inflammatory response to the administered vaccine and were not considered adverse; all effects were resolved by end of recovery period, D59. VRC H1 ssF-3928 mRNA-LNP was generally well-tolerated. In addition, H1ssF_3928 mRNA-LNP elicited binding antibodies in rats.

An investigational influenza H2 vaccine, the HA-F A/Sing protein vaccine (VRC-FLUNPF081-00-VP), which has the same ferritin structure as H1ssF_3928 fused with an influenza virus H2 HA insert to form a nanoparticle displaying eight HA trimers from A/Singapore/1/57 (H2N2) influenza, was previously evaluated for its safety in New Zealand White rabbits to support the Phase 1 clinical study (VRC IND 17399, NCT03186781). As part of that study, anti-ferritin antibodies in the study animals were evaluated by an anti-ferritin-like particle (FLP) ELISA assay. Among all three study groups, immunized with trivalent influenza vaccine (TIV), monovalent HA-F A/Sing, or a trivalent mixture of HA-F A/Sing, an H1 A/NC/1999 ferritin particle (VRC FLUNPF088-00-VP) and an H5 A/IND/05 ferritin particle (VRC FLUNPF089-00-VP), antibodies to neither rabbit ferritin light nor heavy chain were detected. As expected, antibodies to *H. pylori* ferritin were detected in both the monovalent HA-F A/Sing and trivalent influenza ferritin nanoparticle vaccination groups, but not in the TIV vaccination group. The ferritin moiety contained in the vaccines administered to those study animals was identical in amino acid sequence to that in H1ssF_3928 (NCT03186781).

There is no previous human experience with the H1ssF_3928 mRNA-LNP vaccine; however, SARS-CoV-2, influenza, cytomegalovirus, Zika virus, and rabies virus mRNA vaccines have been administered in clinical trials and show promising results in terms of safety and immunogenicity. mRNA vaccines against influenza A/H10N8 and influenza A/H7N9 were evaluated in two Phase 1 clinical evaluations further described below. [14]

Two doses of IM-administered mRNA influenza A/H10N8 vaccine, evaluated at doses ranging from 25 mcg to 100 mcg were well tolerated and immunogenic. A common side effect was injection site pain, with injection site erythema and swelling occurring less frequently. The incidence of fever was higher after a second dose and increased with increasing dose levels. Solicited systemic side effects included myalgia, fatigue, and headache. A 400 mcg dose administered IM was not well tolerated and was discontinued after receipt of a single dose by the two sentinel subjects. mRNA influenza A/H10N8 vaccine was also administered IM but was associated with high rates of solicited adverse events (AEs). Antibody responses as measured by hemagglutination inhibition (HAI) and microneutralization (MN) assays increased with dosage increases.

Two doses of IM-administered mRNA influenza A/H7N9 vaccine were evaluated at doses ranging from 10 mcg to 50 mcg. Common local solicited side effects included injection site pain. Swelling and injection site erythema occurred less frequently. Common solicited systemic AES were headache, myalgia and arthralgia. Fever was also reported and the frequency only increased following the second dose in the 50 mcg group. Post-second dose HAI and MN geometric mean titers (GMTs) were high across all dose groups.

For both studies of influenza, A/H10N8 and influenza A/H7N9 mRNA vaccines all reported serious adverse events (SAEs) deemed unrelated to study vaccine. There were no adverse events of special interest (AESI) or new onset chronic illnesses reported.

Two SARS-CoV-2 mRNA-LNP vaccines have been approved by the FDA for active immunization for the prevention of COVID-19 disease, with 2 dose regimens, at 30 mcg per dose for the Pfizer BioNTech vaccine (approved for individuals aged 16 years and older) and 100 mcg per dose for the Moderna vaccine (approved for individuals aged 18 years and older). Boosters are recommended at 30 mcg dose for the Pfizer BioNTech vaccine and 50 mcg dose for the Moderna vaccine. The proposed clinical trial will evaluate doses in a similar range (10-50 mcg).

A Phase 1 clinical trial (VRC 321; NCT03814720) using the protein construct, H1ssF_3928 has been conducted [2]. H1ssF_3928 protein was evaluated in adults aged 18 to 70 years. The vaccine was assessed at a single dose of 20 mcg or 2 doses of 60 mcg administered 16 weeks apart. Vaccine was administered via intramuscular injection. The 60 mcg dose group was stratified by age (18 to 40, 41 to 49, 50 to 59, and 60 to 70 years old), and participants were followed through 52 weeks after the last vaccination. Fifty-two healthy adults enrolled in the trial between 1 April 2019 and 9 March 2020. The 28 (54%) female and 24 (46%) male participants had a mean age of 46 years (range, 22 to 68 years). The H1ssF vaccine was well tolerated, with mild reported solicited local and systemic reactogenicity. The only local reactogenicity reported was mild pain and tenderness (n = 10, 19%). Systemic reactogenicity most commonly included mild headache (n = 10, 19%) and malaise (n = 6, 12%). There were AEs attributed to vaccination including mild lymphopenia, moderate neutropenia, and one report of abnormal dreams. All AEs self-resolved without sequelae. There were no SAEs or study pauses in the trial. There were four

cases of influenza-like illness in the 60 mcg dose participants: one case in the 18- to 40-year cohort (laboratory-confirmed negative for influenza) and three in the 51- to 60-year cohort (two laboratory-confirmed positive for H1 influenza and one confirmed negative). To address the theoretical possibility of the *H. pylori* ferritin nanoparticle generating an immune response against human ferritin proteins, antibody responses to the human ferritin heavy and light chain, as well as responses to *H. pylori* ferritin, were assessed by enzyme-linked immunoassay (ELISA) in an exploratory objective. Immune responses to *H. pylori* ferritin were observed. However, no antibody responses were seen against either human ferritin antigen after vaccination that increased over the range of vaccine-naïve controls.

Neutralizing antibody responses to the H1ssF_3928 protein nanoparticle vaccine were assessed using a pseudotyped lentivirus neutralization assay. The pseudotyped virus expressed the homologous influenza A/New Caledonia/20/1999 H1 HA and the luciferase reporter gene. Neutralization was quantified by relative decrease in luciferase activity compared with infection of cells in the absence of sera. The 80% inhibition serum titer (IC_{80}) relative to the signal in the absence of sera was calculated. The H1ssF_3928 protein nanoparticle vaccine was immunogenic, resulting in increased neutralizing antibody titers against the homologous A/New Caledonia/20/99 (H1N1) virus. In the single 20 mcg dose group, the geometric mean neutralizing antibody IC_{80} titers increased from baseline to two weeks after vaccination. In the 60 mcg dose group, following the first dose all age groups displayed increased titers at two weeks post prime compared to baseline. These increased neutralizing antibody titers remained elevated over baseline levels following the boost vaccination at Week 16. Moderna conducted a Phase 1/2 study of a quadrivalent seasonal influenza vaccine candidate, mRNA-1010 (NCT04956575). Quadrivalent mRNA-1010 encodes for the hemagglutinin (HA) protein from four seasonal influenza viruses based on the recommendations of the World Health Organization (WHO), including seasonal influenza A/H1N1, A/H3N2 and influenza B/Yamagata- and B/Victoria-lineages. mRNA-1010 was evaluated at 50 mcg, 100 mcg and 200 mcg dose levels in younger adult (age 18-49) and older adult (age 50+) cohorts. No significant safety findings were observed through Day 29. Adverse reactions (ARs) were generally reported more frequently in younger adults compared to older adults, and at higher dose levels. The most common solicited local ARs for both age groups included pain and axillary swelling/tenderness. The most common solicited systemic ARs for both age groups included fatigue, arthralgia, myalgia and headache. Participants receiving a 50 mcg dose achieved robust responses to the influenza A antigens and lower doses including a 25 mcg dose are being evaluated in a Phase 2 study.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

H1ssF_3928-mRNA vaccine

This is a first-in-human study of H1ssF_3928-mRNA vaccine. AEs that have been reported with similar mRNA-LNP vaccines include injection site reactions as well as general systemic AEs. Injection site reactions include pain, tenderness and swelling of the lymph nodes on the same arm of the injection; swelling (hardness) and redness at the injection site. General side effects include fatigue, headache, muscle pain, joint pain, chills, nausea and vomiting, fever and feeling unwell. There is a remote chance that an mRNA-LNP vaccine could cause a severe allergic reaction occurring within a few minutes to one hour after getting a dose of vaccine. Subjects who

have known hypersensitivity or allergy to any components of the study vaccine (including polyethylene glycol [PEG]), or who have a history of a severe reaction following previous immunization with an investigational, authorized, or approved influenza vaccine or mRNA or LNP-containing vaccine will be excluded from study enrollment. Signs of a severe allergic reaction can include difficulty breathing, facial and throat swelling, tachycardia, total body rash, dizziness and weakness. Myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the lining outside the heart) have occurred in some people who have received mRNA-LNP COVID-19 vaccines (Pfizer-BioNTech and Moderna). These AEs have occurred particularly in adolescents and young adults after the second dose. In a population-based cohort study the incidence of acute myocarditis was noted to be 5.8 per 1 million individuals after the second dose [15]. Myocarditis reporting rates among males 12-29 years of age have been noted to be 40.6 cases per million second doses of mRNA COVID-19 vaccines. [16] In most of these people, symptoms began within a few days following receipt of the second dose of the vaccine. The chance of having this occur is low. Symptoms include chest pain, shortness of breath and palpitations. Available data from short-term follow-up suggest that most individuals have had resolution of symptoms, but information is not yet available about potential long-term sequelae.[17-21] In addition, there may be other possible unknown side effects of mRNA-LNP vaccines. Serious and unexpected side effects may also occur. Should a severe allergic reaction occur immediately following vaccination, medications to treat such a reaction will be immediately available for administration according to standard guidelines at the study site.

There is a theoretical risk that immunization with an immunogen containing *H. pylori* ferritin could expand cross-reactive antibodies against human ferritin. Cross-reactive anti-ferritin antibodies have not been observed in animals immunized with constructs containing *H. pylori* ferritin. Study subjects receiving H1ssF_3928 mRNA Vaccine will be monitored for serum iron, ferritin, and anti-ferritin antibodies.

In the VRC 321 Phase 1 clinical trial of the H1ssF_3928 protein, detailed analysis of subjects' iron and ferritin levels did not reveal any product-related trends of clinical concern. Physiologic, expected variations in iron and ferritin values collected during the study did not result in gradable AEs, and there were no AEs of anemia evaluated as related to study product.

In laboratory animals, abnormalities associated with increased clotting were observed following administration of the H1ssF_3928 protein. Study subjects receiving H1ssF_3928 mRNA Vaccine will be monitored for prothrombin time (PT), activated partial thromboplastin time (APPT), and fibrinogen levels.

Quadrivalent inactivated influenza (IIV4) vaccine

IIV4 risks include minor problems such as soreness, redness, swelling, or pain at the injection site, hoarseness, sore, red or itchy eyes, cough, fever, body aches, headache, itching, fatigue, all of which usually occur within 1-2 days of vaccination and are self-limiting. More serious problems including a small increased risk of Guillain-Barré Syndrome, estimated at 1 or 2 additional cases per million people vaccinated, can occur. In addition, IIV4 rarely can cause a severe allergic reaction, or anaphylaxis, which is estimated at ~1 in one million doses of IIV4 administered. The IIV4 to be administered in this study, Fluzone Quadrivalent, is contraindicated for individuals with a history of a severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or to a previous dose of any influenza vaccine (https://www.fda.gov/media/119856/download. Accessed 03 November 2022). Subjects

with a prior history of severe allergic reaction after a previous dose of any influenza vaccine or to a vaccine component, including egg protein, will be excluded from study enrollment. Should a severe allergic reaction occur immediately following vaccination, medications to treat such a reaction will be immediately available for administration according to standard guidelines at the study site.

General Vaccine Risks

Subjects will be counseled on possible side effects following vaccination and followed closely in the immediate post-vaccination period and during the following week for assessment of moderate to severe local or systemic reactogenicity.

Some people get severe pain in the shoulder and have difficulty moving their arm after a shot has been given. This happens very rarely and can be prevented by assuring that the IM injection is done in the appropriate area of the deltoid muscle.

Syncope (fainting) can occur in association with administration of injectable vaccines. In the immediate post-vaccination period, all subjects will be monitored in a sitting or lying position for 30 minutes following vaccination to help prevent fainting, and injuries caused by a fall.

Nasal Absorptive Matrix Sampling

Obtaining a nasal absorptive matrix sample can cause discomfort in the nares, epistaxis, and watery eyes at the time of collection.

Blood Drawing

Drawing blood may cause transient discomfort and fainting. Fainting is usually managed by having the subject lie down. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the draw site for several minutes. Drawing blood may also cause infection. The use of sterile techniques will reduce this risk. Risks for blood drawn may include anemia.

Electrocardiogram (ECG)

The electrodes of an electrocardiogram (ECG) may feel cold when applied; in rare cases, a rash or skin irritation may develop where the patches are placed. This type of irritation usually resolves by itself, but topical medication is occasionally required. Subjects in the study will have one ECG at screening (Days -14 to -1) and a repeat ECG at Study Day 3 ±1 day (Study Visit 2). Additional ECGs will be performed if deemed medically necessary based on reported symptoms.

Risks of Genetic Testing

Genetic data may be stored and shared with other researchers through a controlled-access online repository, such as the database of Genotypes and Phenotypes (dbGaP). There may be a risk that genetic information, while not linked with personally identifiable information (PII), may potentially be re-identified and linked to a subject and could be misused for discriminatory purposes. However, such a risk is perceived as low and state and federal laws provide some protections against genetic discrimination. Researchers who will have access to genetic information will take measures to maintain the confidentiality of the information.

Risk of Loss of Confidentiality

There is also a risk for loss of confidentiality (See Section 10.1.3). To minimize this risk, data will be stored in a password-protected computer database. Samples will be labeled with barcodes prior to storing with a unique identifier. All biological samples will be stored in secure and monitored freezers accessible only to key personnel with the appropriate access. Personnel working on this study will have undergone training for responsible conduct of research with human subjects and are listed on the delegation log/key personnel list and their relevant institutions.

2.3.2 Known Potential Benefits

Participation in this study provides no known direct benefit to the individual subjects.

2.3.3 Assessment of Potential Risks and Benefits

This study may not benefit participants directly. As this is a first-in-human trial, it is unknown whether the study product, H1ssF_3928 mRNA Vaccine, will help to protect subjects from influenza disease caused by H1 subtypes or, if it does, how long that protection may last. The immunogenicity comparator IIV4 may offer some protection against influenza infection for the strains of influenza contained in the vaccine.

Study risk is minimized by using a dose escalation structure, as well as a sentinel group approach within each dose level. Group safety reviews occur between sentinel and expanded groups, and among dose groups.

While individual subjects may not benefit from involvement in this study, their participation is crucial to the larger societal benefit of improved medical interventions. By participating in this study, subjects are enabling the development of a potential advancement to address whether broader protection against H1 influenza subtypes and related subtypes are afforded by the H1ssF_3928-mRNA Vaccine.

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
Primary	
To assess the safety of a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults (18-49 yrs.) at doses of 10 mcg, 25 mcg, and 50 mcg	Number and percentage of participants experiencing solicited local and systemic reactogenicity adverse events (AEs) through 14 days. Number and percentage of participants experiencing any unsolicited AEs through 28 days. Number and percentage of participants experiencing any medically-attended adverse events (MAAEs), new-onset chronic medical

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
	conditions (NOCMCs), adverse events of special interest (AESIs) corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, influenza-like illnesses (ILIs), and SAEs through one year (Day 366) and AESIs corresponding to myocarditis, pericarditis, or myopericarditis through Day 57 Number and percentage of participants experiencing clinical laboratory AEs through 57 days (Day 57)
Secondary	
To assess serum antibody response to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.	GMT of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group. Geometric mean fold rise (GMFR) from baseline (Day 1) of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group. GMT of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group. GMFR from baseline (Day 1) of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 8, 15, 29,

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
	and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group. GMT of anti-stalk serum antibodies, measured by enzyme linked immunosorbent assay (ELISA) at Days 1, 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group GMFR from baseline (Day 1) of anti-stalk serum antibodies, measured by ELISA, at Days 8, 15, 29, and 57 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
*Exploratory	
To assess the anti-ferritin antibody response (to human and *H. pylori* ferritin) to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg.	Measures of anti-ferritin antibody response (to human and *H. pylori* ferritin) to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg at Days 1, 29, and 181 by analysis group and stratified by prior season's receipt and/or subsequent receipt (where applicable) of seasonal influenza vaccine within each analysis group
To assess the durability of the immune response to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.	GMT of homologous H1-specific neutralizing antibodies in a reporter microneutralization assay at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group GMT of homologous H1-specific neutralizing antibodies in a viral microneutralization assay at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt and/or

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
	subsequent receipt of seasonal influenza vaccine within each analysis group GMT of anti-stalk serum antibodies, measured by ELISA, at Days 85, 181, and 366 by analysis group and stratified by prior season's receipt and/or subsequent receipt of seasonal influenza vaccine within each analysis group
To assess antibody breadth to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4	Neutralizing antibody response to divergent H1 influenza virus isolates and other Group 1 non-H1 influenza virus isolates at Day 1 and 29 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group Anti-stalk serum antibody response to divergent H1 influenza virus isolates and other Group 1 non-H1 influenza virus isolates at Day 1 and 29 by analysis group and stratified by prior season's receipt of seasonal influenza vaccine within each analysis group
To assess the B cell and/or T cell, including T follicular helper cell, responses to a single dose of VRC H1ssF_3928 mRNA-LNP vaccine administered IM in healthy adults at doses of 10 mcg, 25 mcg, and 50 mcg in comparison to a standard dose of IIV4.	Exploratory immunogenicity evaluations may include B cell assays, T cell assays, and T follicular helper cell assays at selected time points in the entire study population or subsets of participants by analysis group and stratified by prior season's receipt and/or subsequent receipt (where applicable) of seasonal influenza vaccine within each analysis group

*Assays for exploratory endpoints may be performed and the data provided as described in Section 9.4, if available from the research laboratory.

4. STUDY DESIGN

4.1 Overall Design

This is a Phase 1, single-site, open-label, comparator-controlled dose escalating study of an IM-administered mRNA-LNP vaccine encoding for VRC H1ssF_3928 of up to 50 healthy adult volunteers aged 18 to 49 years, inclusive. This study is designed to assess the safety and immunogenicity of one dose of H1ssF_3928 mRNA Vaccine. Eligible participants will be sequentially enrolled into dosing groups (10 mcg, 25 mcg, and 50 mcg, selected optimal dose) to receive the H1ssF_3928 mRNA Vaccine at the specified dose. A separate group of 10 participants will receive licensed quadrivalent influenza vaccine (IIV4). Subjects receiving IIV4 will be followed for safety but only their immune responses will be compared to those of participants receiving H1ssF_3928 mRNA Vaccine. Dosing of H1ssF_3928 mRNA Vaccine will commence at the lowest dose (10 mcg) and only escalate to the next highest dose if safety concerns are not identified. An optimal dosing group will be selected based on safety outcomes (see **Section 4.2**). An overview of the dosing groups is presented in **Table 1 Section 1.1**.

For each VRC H1ssF_3928 mRNA-LNP dosing group, the first two subjects enrolled will be considered the sentinel subgroup **Table 1 Section 1.1**. After the two subjects in the Low Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a review of the reactogenicity and safety data collected through Day 3 for both subjects. This review will be conducted by a safety review committee (SRC), as indicated and detailed in **Section 10.1.6**. Approval by the reviewing group will allow for continued enrollment of the remaining 8 Low Dose Group subjects (expanded subgroup) to complete enrollment of 10 participants. After Low Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 for all Low Dose Group subjects. Approval by the SRC will allow dose escalation and initiation of enrollment of the Medium Dose Group sentinel subgroup.

After the two subjects in the Medium Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the Low Dose Group. Approval will allow for continued enrollment of the Medium Dose Group subjects (expanded subgroup) to complete enrollment of 10 participants. After the Medium Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and adverse event information through Day 7 and clinical laboratory results through Day 8 for all Medium Dose Group subjects. Approval by the SRC will allow dose escalation and initiation of enrollment of the High Dose Group sentinel subgroup.

After the two subjects in the High Dose sentinel subgroup are enrolled and given their vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the Low and Medium Dose Groups. Approval will allow for continued enrollment of the High Dose Group subjects (expanded subgroup) to complete enrollment of 10 participants. After the High Dose Group enrollment is completed, enrollment will be stopped pending an SRC review of the reactogenicity and adverse event information through Day 7 visit and clinical laboratory results through Day 8 for all High Dose Group subjects.

Reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 from the first three dosing groups will guide the SRC during their review meeting to the selection of an optimal dose group to include an additional 10 subjects who will receive the optimal dose of mRNA-LNP (see **Section 4.2**). Vaccinations will be performed by licensed vaccine administrators while assessments will be performed by the clinical team.

For immunogenicity comparison, a separate group of 10 participants will receive IIV4. Enrollment of participants into the IIV4 group is limited to when the vaccine is available for a given year. Concurrent enrollment of the IIV4 group and any pre-specified study product group or groups presents logistical challenges due to the availability of the IIV4 vaccine and may preclude enrollment of all study participants prior to the subsequent off-season.

All subjects will complete a subject memory aid for 14 days after study vaccination. Solicited AEs will be collected through 14 days after vaccination and will be reviewed at Visits 1 - 4, Day 15. Unsolicited, non-serious AEs and SAEs will be collected through 28 days after the vaccination and will be reviewed at Visits 1 - 5, Day 29. MAAEs, NOCMCs, AESIs corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, influenza-like illnesses (ILIs), SAEs, and ILIs will be collected from the time of vaccination throughout the study period. AESIs corresponding to myocarditis, pericarditis, or myopericarditis will be collected from the time of vaccination through Day 57 Subjects' participation will be approximately 12 months. **Table 2** provides a detailed outline of the study schedule.

Blood samples and nasal specimens will be obtained at specified time points following vaccination for safety laboratory assessments and assessments of innate and adaptive immune responses.

Table 3. Blood Volume Collection Summary

Days relative to 1st vaccination	**-14 to -1**	**1**	**3**	**8**	**15**	**29**	**57**	**85**	**181**	**366**
Study visit	00	01	02	03	04	05	06	07	08	09
Vaccination visit		X								
Laboratory tests and blood volumes (mL)										
Screening labs	30	0	0	0	0	0	0	0	0	0
Safety labs[2]	0	20	4	16	0	16	16	0	0	0
Serologic assays	0	20	20	20	20	20	20	20	20	20
Cellular assays	0	40	0	40	24	24	24	40	40	24
Cellular assays-secondary research	0	24	0	24	40	40	40	24	24	40
RNA assays-secondary research	0	5	5	5	0	0	0	0	0	0
Total volume per visit	30	109	29	105	84	100	100	84	84	84

Cumulative total	30	139	168	273	357	457	557	641	725	809

[1]Additional blood draws may be needed as part of follow up of AEs or at the discretion of the PI when related to subjects' safety.

[2]Safety labs include: hematology, serum chemistry, and coagulation studies.

Schedule of assessments can be found in **Section 1.2, Schedule of Activities**

Protocol Schema can be found in **Section 1.1, Study Schema**.

Dose escalation or dose-ranging details are found in **Section 6.1.2, Dosing and Administration**

Full details of interim analysis are found in **Section 9.4.6, Planned Interim Safety Analysis**

4.2 Scientific Rationale for Study Design

For this Phase 1 first-in-human trial, a conservative dose escalation design will be used, in which each dose group of 10 vaccine recipients, is split into two subgroups. The first subgroup, termed the sentinel subgroup, includes 2 vaccine recipients. The second subgroup, termed the expanded subgroup, includes the remaining 8 vaccine recipients of dose group. In each of the three initial (10 mcg, 25 mcg, and 50 mcg) dose groups, enrollment is halted after vaccination of the sentinel subgroup. This allows the opportunity to identify early vaccine related AEs in this subgroup prior to vaccinating additional subjects, a design that decreases the risk of multiple serious vaccine-related AEs compared to sequential vaccination of the entire dose group without halting [22]. After assessing safety of the sentinel subgroup, enrollment is expanded to the remaining 8 subjects in the group. Escalation to the next dose group will be delayed until reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 following the vaccination is available from all subjects in the preceding dose group. An optimal dose will be selected based on safety outcomes from the 10 mcg, 25 mcg, and 50 mcg dosing groups. For the optimal dose, the highest dose with no identified safety concerns as determined by the SRC will be selected. This approach will allow determination of an optimal dose which will be administered to 10 additional participants. In total 20 subjects will receive the optimal dose and a separate group of 10 participants will receive active IIV4 vaccine as an immunogenicity comparator (open-label). Active immunogenicity-comparator recipients are included in order to best compare the magnitude and breadth of H1 stalk antibody response. The safety and tolerability profile for IIV4 vaccine is well established.

4.3 Justification for Dose

The IM route of administration has been used for other mRNA-LNP investigational products and has more recently been extensively used for administration of SARS-CoV-2 mRNA vaccines. Selected dosage levels of 10 mcg, 25 mcg and 50 mcg are based on clinical experience with other mRNA-LNP investigational, authorized and approved vaccines. A study of influenza A/H10N8 mRNA vaccines determined that 400 mcg doses were not well tolerated while doses of 100 mcg or less were acceptable. A clinical study of A/H7N9 mRNA vaccine evaluated the vaccine at similar dosage levels. On August 23, 2021, US Food and Drug Administration (FDA) approved the Pfizer/BioNTech mRNA COVID-19 vaccine for use in persons 16 years of age and older for the prevention of COVID-19 disease caused by SARS-CoV-2. On January 31, 2022, FDA approved the Moderna mRNA COVID-19 vaccine for use in persons 18 years of age or

older for the prevention of COVID-19 disease caused by SARS-CoV-2. The proposed clinical trial will evaluate doses in a similar range (10-50 mcg).

An Investigational New Drug application (IND)-enabling *in vivo* repeat dose toxicity study was conducted in Wistar Han rats utilizing an accelerated vaccination schedule involving three doses of VRC H1ssF_3928 mRNA-LNP produced from the engineering run used in the manufacturing process for current Good Manufacturing Practice (cGMP) production. VRC H1ssF_3928 mRNA-LNP was administered by IM injection at Day 1, Day 15, and Day 29. Each 50 mcg dose was administered in a volume of 0.1 mL. The 3-dose vaccination schedule represents n+2 for the proposed single dose schedule to be administered during this Phase 1 clinical trial. There were no VRC H1ssF_3928 mRNA-LNP-related unscheduled deaths. VRC H1ssF_3928 mRNA-LNP-related clinical observations included limited use of the hind limbs, which was not considered adverse due to reversibility and lack of systemic health effects.

VRC H1ssF_3928 mRNA-LNP treatment resulted in positive erythema or eschar score and edema at the injection site. Edema increased in severity at each consecutive dose and generally resolved entirely within 72 hours. The positive edema scores were not considered adverse as they were reversible.

VRC H1ssF_3928 mRNA-LNP-related body weight effects were noted for a few days following each dose administration compared to control animals. Decrease in body weight correlated with clinical observations of limb function impairment, edema, and/or erythema and a reduction in food consumption. As limb impairment and positive Draize scoring resolved, so did the body weight and food consumption values.

VRC H1ssF_3928 mRNA-LNP-related increase in body temperature was noted following each dose administration which generally resolved within 72 hours. On Days 3 and/or 31, males and females had minimal to mild increases in mean white blood cells (WBC), characterized by an increase in mean neutrophils, large unstained cells (LUC), and/or eosinophils and monocytes. These changes are indicative of an inflammatory response and resolved by Day 59. On Days 3 and 31, males and females had mild to moderate decreases in mean reticulocytes—these changes resolved by Day 59. On Days 3 and/or 31, males and females that were administered VRC H1ssF_3928 had minimal to mild decrease in mean platelets. These changes were resolved by Day 59. On Days 3 and/or 31, males and females administered VRC H1ssF_3928 had minimal to mild prolongations in mean prothrombin time (PT) and/or activated partial thromboplastin time (APTT). On Days 3 and 31, males and females had mild to moderate increases in mean fibrinogen, indicating an inflammatory response. All changes resolved by Day 59.

On Days 3 and/or 31, males and females had minimal to mild decreases in mean cholesterol and/or triglyceride, and these changes were resolved by Day 59. On Days 3 and 31, males and females also had minimal to mild decreases in mean albumin and albumin/globulin (A/G) with minimal to mild increases in mean globulin. Associated with the decrease in mean albumin was a decrease in mean calcium on Day 3 in females given VRC H1ssF_3928 mRNA-LNP. These collective changes indicate an inflammatory response, and they resolved by Day 59. On Days 3 and 31, males and females in all groups had minimal to moderate decreases in mean alkaline phosphatase (ALP). These changes were not resolved by Day 59 and were considered related to diminished food consumption in all groups and procedure-related.

VRC H1ssF_3928 mRNA-LNP -related organ weight changes consisted of higher spleen weights, lower thymus weights, and higher liver weights in males and females. Significant

mildly more elevated spleen weights correlated with microscopic increased cellularity of the red and white pulp. Significant minimally lower absolute and relative thymus organ weights correlated with microscopic decreased lymphoid cellularity. Minimally higher absolute and relative liver weights correlated with microscopic minimal to mild periportal hepatocyte vacuolation in males and females. These weight changes were not present in animals during the recovery period on Day 59.

There were no VRC H1ssF_3928 mRNA-LNP related macroscopic findings. Test article-related microscopic findings were present in males and females at injection sites, draining lymph nodes, sciatic nerve, spleen, bone marrow, thymus, and liver. All changes were either partially or wholly reversed following the recovery period. Findings at the injection sites were considered locally poorly tolerated based on the local inflammatory reaction and epidermal single cell necrosis and/or erosion/ulcer. The local inflammatory reaction resulted in neutrophilic/mononuclear increased cellularity in the spleen, bone marrow, and draining lymph nodes (hematology correlates of increased WBC and neutrophils).

In conclusion, VRC H1ssF_3928 mRNA-LNP was generally well-tolerated in rats. The No-Adverse-Effect-Level (NOAEL) was 50 mcg/dose/day. These results indicate that the vaccine test article demonstrates an acceptable pre-clinical safety profile at the planned maximum dose level of 50 mcg. In addition, the VRC H1ssF_3928 mRNA-LNP elicited robust antibody responses in rats. Binding antibodies to both the H1ssF_3928 protein and recombinant full-length HA from influenza A/New Caledonia/20/1999 (H1N1) were detected in rat sera collected on study Days 22 and 31, by ELISA.

In summary, based on prior clinical experience with other mRNA vaccines and toxicology studies we do not anticipate significant safety signals. The antigen doses to be evaluated in this Phase 1 trial of VRC H1ssF_3928 mRNA-LNP are 10 mcg, 25 mcg and 50 mcg.

5. STUDY POPULATION

This study will enroll healthy adult volunteers from the general population who meet eligibility criteria.

Eligibility

A potential participant is eligible to join this trial if he/she meets all the following inclusion criteria and none of the following exclusion criteria. Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572. No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies.

5.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provide written informed consent prior to initiation of any study procedure.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Are males or non-pregnant, non-breastfeeding females, 18 to 49 years of age, inclusive at time of screening and enrollment.

4. Must agree to collection of venous blood and nasal absorption specimens per protocol and enrollment in DMID Protocol No. 19-0025 biorepository protocol for secondary research and use of residual biologic specimens.

5. Are in good health[1] and do not have clinically significant medical, psychiatric, chronic or intermittent health conditions including those listed in Exclusion Criteria (**Section 1**).

   *[1] As determined by medical history, medication use, and physical examination to evaluate ongoing chronic medical or psychiatric diagnoses or conditions, defined as those that have been present for at least 90 days, which would not affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. These medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, emergency room [ER] or urgent care for condition [excluding musculoskeletal conditions]), or invasive medical procedure and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose or in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening or treatment of continued symptoms of medical diagnosis or condition. Note: Low dose topical corticosteroids as outlined in the Subject Exclusion Criteria (see **Section 1**) as well as herbals, vitamins and supplements are permitted.*

6. Does not have an ongoing symptomatic condition[2] for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan.

   [2] e.g., ongoing fatigue without a diagnosis for symptom.

7. Pulse is 50 to 100 beats per minute, inclusive.

8. Systolic blood pressure is 90 to 140 mmHg, inclusive.

9. Diastolic blood pressure is 55 to 90 mmHg, inclusive.

10. Body mass index (BMI) of 18 kilograms/square meter (kg/m^2) (inclusive) to <35 kg/m^2 at screening

11. Women of childbearing potential[3] must agree to use or have practiced true abstinence[4] or use at least 1 acceptable primary form of contraception.[5,6]

    [3] Not of childbearing potential - post-menopausal females (defined as having a history of amenorrhea for at least one year) or a documented status as being surgically sterile (hysterectomy, bilateral oophorectomy, salpingectomy, or Essure® placement with history of documented radiological confirmation test at least 90 days after the procedure).

    [4] True abstinence is 100% of time no sexual intercourse (male's penis enters the female's vagina). (Periodic abstinence [e.g. calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception).

    [5] Acceptable forms of primary contraception include monogamous relationship with a vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the study product, tubal ligation, intrauterine devices, birth control pills, and injectable/implantable/insertable hormonal birth control products.

[6] Must use at least one acceptable primary form of contraception or true abstinence for at least 30 days prior to receipt of study product and at least one acceptable primary form of contraception or true abstinence for at least 30 days following receipt of study product.

12. Women of childbearing potential[3] must have a negative serum HCG pregnancy test at screening and a negative urine HCG pregnancy test within 24 hours prior to the study vaccination.
13. Male participants receiving VRC H1ssF_3928 mRNA-LNP must agree to refrain from donating sperm and to use contraception until Day 60 after vaccination.[7, 8]

    [7] Acceptable contraception includes abstinence from intercourse with a female of childbearing potential or use of a male condom when engaging in any activity that allows for passage of ejaculate to a female during the intervention period for at least 60 days after study vaccination.

    [8] Males in the immunogenicity comparator group do not have to refrain from sperm donation or abstain from intercourse or agree to use a male condom for purposes of this study.

14. Must have received at least one licensed seasonal influenza vaccine within the previous 5 seasons.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Have an acute illness[9] or fever (body temperature ≥ 38.0°C/100.4°F), as determined by the site Principal Investigator (PI) or appropriate sub-investigator, within 72 hours prior to study vaccination.

   [9] An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.

2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation.[10]

   [10] Including acute, subacute, intermittent or chronic medical disease or condition that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this trial.

3. Has a screening laboratory[11] >Grade 1.

   [11] WBC count, absolute neutrophil count, absolute lymphocyte count, hemoglobin, platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, lipase

4. Has a positive urine toxicology screen (i.e., non-prescribed amphetamines, cocaine, and opiates).
5. ECG is deemed to be clinically significant by the PI or appropriate sub-investigator.

6. Total iron binding capacity, iron, ferritin and troponin (hsTnI) outside the laboratory normal range at screening.
7. Have any known or suspected immunosuppressive condition, acquired or congenital, or autoimmune conditions as determined by history and/or physical examination.
8. Have immunosuppression as result of treatment including a recent history (within 6 months prior to administration of study vaccine) or use of immunosuppressive or other immune-modifying drugs.
9. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
10. Have known active or recently active (12 months) neoplastic disease or a history of any hematologic malignancy. Non-melanoma, treated, skin cancers are permitted.
11. Have known human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection at screening.
12. Have a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or HIV types 1 or 2 antibodies at screening.
13. Have known chronic liver disease, including fatty liver disease.
14. Have a known allergy or severe reaction to any components of the study vaccine (including PEG) or the seasonal influenza vaccine (including egg protein).
15. Have a history of a severe reaction following previous immunization with an investigational, authorized, or approved influenza vaccine or mRNA or LNP-containing vaccine.
16. Have a history of Guillain-Barré Syndrome.
17. Have a known history of myocarditis or pericarditis.
18. Have a history of alcohol or drug abuse within 3 years prior to study vaccination.
19. Have any diagnosis, current or past, of schizophrenia, bipolar disease or other psychiatric diagnosis that may interfere[12] with subject compliance or safety evaluations.

    [12] *As determined by the site PI or appropriate sub-investigator.*
20. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination.
21. Have taken oral or parenteral (including intra-articular) corticosteroids of any dose within 30 days prior to study vaccination. Intranasal or topical (skin or eyes) corticosteroids are permitted.
22. Have taken high-dose inhaled or nebulized corticosteroids[13] within 30 days prior to study vaccination.

    [13] *High-dose defined as per age as using inhaled high-dose per reference chart in the National Heart, Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma (EPR-3) or other lists published in UPTODATE.*
23. Have any significant disorder of coagulation requiring ongoing or intermittent treatment.

24. Have received seasonal influenza vaccine within the 90 days prior to enrollment or plans to receive a seasonal influenza vaccine 60 days after study vaccination.
25. Have received any approved or authorized vaccines other than seasonal influenza vaccine within 60 days before enrollment or plans to receive an approved or authorized vaccine 60 days after study vaccination.
26. Have a known history of documented influenza infection within the past 90 days.
27. Have a history of receipt of an investigational H1 influenza vaccine within the past 10 years.
28. Have a history of receipt of a ferritin-based vaccine or an investigational H5 influenza vaccine.
29. Previous participation in DMID Protocol No. 18-0010 human influenza challenge study.
30. Received immunoglobulin and/or any blood products (except Rho D immunoglobulin) within the 90 days prior to study vaccination.
31. Received an experimental agent[14] within 60 days prior to the study vaccination or expect to receive another experimental agent during the trial-reporting period.[15]

    [14] *Including vaccine, drug, biologic, device, blood product, or medication.*

    [15] *Other than from participation in this trial*

32. Are participating or plan to participate in another clinical trial with an interventional agent[16] that will be received during the trial-reporting period[17].

    [16] *Including licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication.*

    [17] *Approximately 12 months after the first study vaccination.*

33. The subject has any abnormality or permanent body art (e.g., tattoo) that, in the opinion of the investigator or appropriate sub-investigator, would obstruct the ability to observe local reactions at the injection site.
34. Donation of blood or blood products within 30 days prior to dosing and plans to donate within 60 days following dosing.
35. Has had significant exposure to someone with laboratory-confirmed SARS-CoV-2 infection or influenza in the 14 days prior to screening or during the period between screening and enrollment visit[18].

    [18] *Defined by the Centers for Disease Control and Prevention (CDC) as a close contact with someone who has COVID-19.*

36. Has had a positive SARS-CoV-2 test (home or laboratory-based) within 14 days prior to the screening visit or during the period between screening and enrollment visits.

5.2.1 Exclusion of Specific Populations

This is a first-in-human study, to be conducted in a healthy adult population. There is no prior clinical experience with the VRC H1ssF_3928 mRNA-LNP vaccine or novel ionizable cationic lipid. We are excluding adults 50 years and older, children and adolescents under 18 years of

age, pregnant women, women who are planning to become pregnant within 30 days of vaccination, and women who are breast-feeding.

5.3 Inclusion of Vulnerable Participants

Not Applicable

5.4 Lifestyle Considerations

During this study, participants are asked to:

- For women of child-bearing potential, avoid getting pregnant during the study from the time of screening until 30 days after the study vaccination.
- For women of child-bearing potential, use an acceptable contraception method or true abstinence from 30 days before the study vaccination until 30 days after the study vaccination.
- For sexually active males, abstain from intercourse with a female partner or use a medically acceptable form of contraception or true abstinence, until day 60 after the vaccination. Males in the immunogenicity comparator group do not have to abstain from intercourse or use contraception.
- If male, refrain from donating sperm until Day 60 after vaccination. Males in the immunogenicity comparator group do not have to refrain from sperm donation.
- Refrain from receiving another experimental agent or participating in another clinical trial with an interventional agent within 60 days before study vaccination through the entire study period.
- Refrain from receiving the seasonal influenza vaccine within 90 days prior to enrollment and within 60 days of the study vaccination.
- Refrain from receiving any approved or authorized vaccines within 60 days (before or after) study vaccination.
- Refrain from donating blood within 30 days prior to receiving the study vaccine and within 60 days following receipt of the study vaccine.

5.5 Screen Failures

Following consent and screening evaluations, the investigator or designee will review the inclusion/exclusion criteria and determine the subject's eligibility for the study and administration of the study product.

Subjects who are found to be ineligible will be told the reason for ineligibility. Subjects who are ineligible and not administered the study product will be replaced. Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) but before administration of the study product may be replaced.

Only the following information will be collected on screen failures: demographics (age, screen number, sex, ethnicity, and race) and reason for ineligibility.

For individuals who do not meet the criteria for participation in this study (screen failure) because of a laboratory value that is outside the range of eligibility, but the laboratory value is thought to be due to an acute condition or due to laboratory error, the abnormal laboratory assessment may be repeated once. Similarly, for vital signs that fall outside of the range of eligibility, but the vital sign is thought be due to an acute condition or measurement error, the vital sign may be repeated once. If the screening ECG is determined to be clinically significant, the ECG will not be repeated and the individual will be excluded.

5.6 Strategies for Recruitment and Retention

5.6.1 Recruitment

Potential participants will be selected from existing participant registries, through advertising or by word of mouth in the Duke University, Durham, NC area and surrounding communities. Recruitment staff will contact interested individuals to assess their eligibility for this study. It is anticipated that the demographic composition of subjects for this study will be representative of the respective region.

The Duke University Health System Institutional Review Board (IRB) will approve the recruitment process and all materials to be provided for recruitment or to any prospective subjects directly.

Screening will begin with a brief discussion about the study between the study staff and potential study subjects. Information about the study will be presented to potential subjects and questions will be asked to determine potential eligibility. Some people will be excluded from the study based on demographic data and medical history (i.e., pregnant, prior vaccination, etc.) A Health Insurance Portability and Accountability Act (HIPAA) waiver of authorization will also be obtained to review medical records for the purposes of screening activities. Other subjects may be excluded based on medical history available in the medical record. Study procedures will begin only after informed consent is obtained.

5.6.2 Retention

Retention of subjects in this trial is especially important for determining the primary and secondary endpoints. As such, after vaccination, participating subjects will be reminded of subsequent study visits and every effort will be made to accommodate the subject's schedule to facilitate follow-up within the specified visit window. Additionally, there are many circumstances that influence the ability to obtain outcome information after vaccination. Follow-up visits may be conducted by phone or IRB-approved telehealth means if in-person visits are not feasible. Lastly, compensation strategies will be implemented to encourage subjects to return for follow-up assessments and sample collections.

5.6.3 Compensation Plan for Subjects

Compensation will be determined locally and in accordance with IRB requirements, and subject to IRB approval. Compensation will be described in the site-specific informed consent.

5.6.4 Costs

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial.

6. STUDY PRODUCT

6.1 Study Product(s) and Administration

6.1.1 Study Product Description

Product 1: H1ssF_3928 mRNA-LNP Vaccine

The investigational vaccine consists of modified nucleoside messenger RNA (mRNA) encapsulated in LNP, comprised of four lipid components: (Bis(2-butyloctyl) 10-(N-3-(pyrrolidin-1-yl)propyl)nonanamido)nonadecanedioate [ionizable lipid], DSPC, cholesterol, and PEG lipid). These lipid components are similar to those used in the Moderna and Pfizer SARS-CoV-2 vaccines, except the novel ionizable cationic lipid, for which there is not yet clinical experience.

Product 2: Diluent

The diluent for H1ssF_3928 mRNA Vaccine will be sterile 0.9% sodium chloride for injection, USP.

Product 3: Seasonal Influenza Vaccine (IIV4) Immunogenicity Comparator: Fluzone Quadrivalent

All participants without contraindications will be given injectable IIV4: Fluzone Quadrivalent (Sanofi Pasteur Inc.)[https://www.fda.gov/media/119856/download. Accessed 03 November 2022].

Fluzone Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. It is an inactivated influenza vaccine, prepared from influenza viruses propagated in embryonated chicken eggs.

6.1.2 Dosing and Administration

Group	*Product Name*	*Dose*	*Route*	*Frequency of Administration*	*Duration of Therapy*
Low Dose	H1ssF_3928 mRNA Vaccine	10 mcg	IM	Once	N/A
Medium Dose	H1ssF_3928 mRNA Vaccine	25 mcg	IM	Once	N/A
High Dose	H1ssF_3928 mRNA Vaccine	50 mcg	IM	Once	N/A

Optimal Dose	H1ssF_3928 mRNA Vaccine	Optimal Dose	IM	Once	N/A
Immunogenicity Comparator	IIV4	60 mcg (15 mcg per antigen)	IM	Once	N/A

6.1.3 Dose Escalation

For this Phase 1 first-in-human trial, a conservative dose escalation design will be used, in which each dose group of 10 investigational vaccine recipients will be split into two subgroups. The first subgroup, termed the sentinel subgroup, includes 2 investigational vaccine recipients. In each of the three dose phases (10 mcg, 25 mcg and 50 mcg), enrollment is halted after vaccination of the sentinel subgroup. This allows the opportunity to identify potential early vaccine related AEs in this subgroup prior to exposure of additional subjects, a design that decreases the risk of multiple serious vaccine related AEs compared to sequential vaccination of the entire dose group without halting. [22] After enrollment of the expanded subgroups (8 recipients), escalation to the next dose group will be delayed until reactogenicity and AE information through Day 7 and clinical laboratory results through Day 8 following the vaccination is available from all subjects in the preceding dose group and the SRC has approved. The optimal dose will be the highest dose with no safety impacts.

6.1.4 Dose Modifications

Not applicable

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Product 1: H1ssF_3928 mRNA Vaccine (Duke Human Vaccine Institute [DHVI])

Product 2: Diluent (sterile 0.9% sodium chloride for injection, USP) (Fisher)

Product 3: Seasonal Influenza Vaccine (IIV4) Immunogenicity Comparator (Fisher)

Products 1, 2 and 3 will be distributed by DMID Clinical Material Services (CMS). Records will be maintained that document receipt, release for dosing, disposal, or return to the sponsor.

For product 1, upon request by DMID, study products will be transferred to the following address:

DMID Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

Based on availability, DMID will advise on sourcing additional specific products which may include CMS or site-specific procurement. This includes but may not be limited to, mixing vials, syringes, needles and shipping supplies for this study. The study products will be provided

through the DMID CMS to the participating clinical site prior to the start of this trial upon request and with prior approval from DMID. Should the site PI require additional study products, further instructions will be provided in the protocol-specific Manual of Procedures (MOP).

Accountability: After receipt of the study products, the site PI is responsible for study product distribution and disposition and has ultimate responsibility for study product accountability. The site PI may delegate to the participating site's research pharmacist responsibility for study product accountability. The participating site's research pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, preparation/dilution, dispensation, storage conditions, and final disposition of the study product(s). All study product(s), whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the participating site's study product accountability records and dispensing logs per the site monitoring plan. Refer to the protocol-specific MOP for details on storing study products.

Unused study products will be retained until study conclusion or until accountability via verification of inventory or monitoring has occurred and written notification stating retention is no longer required is received from the Sponsor, as applicable per the site's SOPs.

Accountability may be performed through verification of inventory and during monitoring. DMID does not require used containers of study product to be maintained at the research pharmacy until the clinical monitors have confirmed the disposition of all study products.

Retention of used study product containers for monitoring is only required when the local institution's SOP/policy mandates it. If local SOPs allow/require destruction of used study product containers, then the used vials can be destroyed per the site's SOPs with a second staff member's observation and signed verification (two signatures) that the used vials were destroyed.

Final disposition of the unused study products will be determined by DMID and communicated to the participating sites by the DMID Clinical Project Manager.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Product 1: H1ssF_3928 mRNA Vaccine

The mRNA-LNP is formulated at a concentration of 0.5 mg mRNA/mL in a sterile aqueous buffer consisting of 300 mM sucrose, 102.7 mM NaCl, and 8 mM phosphate. The H1ssF_3928 mRNA vaccine was manufactured under Good Manufacturing Practice (GMP) conditions by two manufacturers. The Duke Human Vaccine Institute GMP Program (DGP) (Durham, NC) manufactured the GMP-grade mRNA and provided it to Evonik Canada (Burnaby, BC, Canada) for the formulation into lipid nanoparticles.

The vaccine is filled into single-dose vials and stored frozen at ≤-60°C.

The vaccine is supplied in single-dose 2 mL Type 1 glass vials. The H1ssF_3928 mRNA Vaccine product is a white to off-white suspension.

Each vial contains 0.7 mL of H1ssF_3928 mRNA, 0.5 mg/mL and is individually labeled with the product name, description, and caution statement. The product will be diluted in normal

saline to achieve targeted doses. The label was affixed prior to final fill with H1ssF_3928 mRNA Vaccine by Evonik Canada, Burnaby, BC, Canada.

Product 2: Diluent

The diluent, sterile 0.9% sodium chloride for injection, USP, or normal saline, is a sterile, nonpyrogenic, isotonic solution; each mL contains sodium chloride 9 mg. It contains no bacteriostatic agent, antimicrobial agent, preservatives, or added buffer and is supplied only in single-dose containers. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5-7.0). Normal saline will be supplied as single-use vials.

Product 3: Seasonal IIV4 Immunogenicity Comparator

The study will utilize licensed seasonal quadrivalent influenza vaccine as a comparator product. The licensed seasonal influenza vaccine will be acquired by DMID CMS with storage and administered in accordance with the manufacturer's recommendations.

6.2.3 Product Storage and Stability

Product 1: H1ssF_3928 mRNA-LNP Vaccine

Study vaccine must be shipped on dry ice. Study vaccine must be stored at ≤-60°C. Vials of the filled drug product can be thawed at room temperature. The undiluted drug product is stable at -USP controlled room temperature for up to 5 days. After dilution in saline, the diluted clinical trial material is stable for up to 6 hours at USP controlled room temperature.

Product 2: Diluent

The normal saline diluent will be stored per manufacturer's instructions. Details are provided in the protocol-specific MOP.

Product 3: Seasonal Influenza Vaccine (IIV4) Immunogenicity Comparator

Licensed seasonal IIV4 will be stored as per manufacturer's recommendations. Details are provided in the protocol-specific MOP.

The temperature of the storage units for study product will be continuously monitored and recorded during the duration of this trial per the participating site's standard operating procedures, and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The research pharmacist must alert the site PI and study coordinator, if the temperature fluctuates outside of the required range.

In the event the temperature fluctuates outside of the required range, the affected study product(s) must not be administered. The site PI or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturers will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine of product are provided in the protocol-specific MOP.

6.2.4 Preparation

H1ssF_3928 mRNA Vaccine

The product is diluted in normal saline. Detailed study product preparation instructions are provided in the protocol-specific MOP.

Seasonal Influenza Vaccine (IIV4) Immunogenicity Comparator

The IIV4 product is prepared per manufacturer instructions. Details are in the protocol-specific MOP.

6.3 Measures to Minimize Bias: Randomization and Blinding

This study is a Phase 1 study to assess the safety and immunogenicity of different doses of the study product. The immunogenicity comparator is a standard IIV4 vaccine. Due to the logistical challenges of concurrently enrolling the immunogenicity comparator cohort and sequential investigational vaccine cohorts in a seasonal influenza vaccine season, randomization and blinding is infeasible. Therefore, the study is not randomized and is open-label.

6.3.1 Treatment Assignment Procedures

Subjects will be assigned to receive either one of three doses of H1ssF_3928 mRNA Vaccine or standard IIV4 vaccine depending upon the cohort into which they enroll.

Table 4: Treatment Assignment

Group	Vaccine Dose Group	Cohort Size
Low Dose	10 mcg	10
Medium Dose	25 mcg	10
High Dose	50 mcg	10
Optimal Dose	Optimal Dose	10
Immunogenicity Comparator	IIV4	10

6.4 Study Intervention Compliance

Subjects will be directly observed at the time of dosing by a member of the clinical research team who is licensed to administer the study product. Administration will be documented on the Treatment Administration Record and entered into the electronic Case Report Form (eCRF).

6.5 Concomitant Therapy

All concomitant medications taken within 90 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility and recorded on the data collection forms/source documentation. Medications reported in the eCRF from the paper CRFs are limited to those taken within 30 days prior to receipt of study product. Concomitant medications will be reviewed at every study visit through the end of the study.

Women of childbearing potential in a heterosexual relationship must agree to use true abstinence or use at least one acceptable primary form of contraception through 30 days following receipt of study product (see Inclusion Criterion #11).

Male participants must agree to refrain from donating sperm and to be abstinent from heterosexual intercourse with a female of childbearing potential as their preferred and usual lifestyle or must agree to use a male condom when engaging in any activity that allows for passage of ejaculate to a female for at least 60 days after receipt of study product unless they are in the seasonal influenza immunogenicity comparator group.

Subjects should not have received seasonal influenza vaccine within 90 days prior to enrollment or within 60 days of study vaccination. Subjects should not have received any approved or authorized vaccines within 60 days prior to study enrollment or 60 days after study product dosing.

Receipt of any influenza vaccine prior to study vaccination for the 2021/2022, 2022/2023, and 2023/2024 influenza vaccine seasons will be documented. Note that subjects should not have received influenza vaccine three months prior to receipt of study product but should have received at least one seasonal influenza vaccine during the past 5 seasons. Receipt of seasonal influenza vaccine subsequent to study vaccination will be documented throughout the study."

For subject receipt of non-seasonal influenza vaccine, including those that are experimental, product type, vaccine virus strains and approximate date will be documented. Note that subjects should not have received an investigational H1 influenza vaccine within the past 10 years. Prior participation in any influenza challenge study will be documented and captured in the clinical database. Previous participation in DMID Protocol No. 18-0010 human influenza challenge study is considered an exclusion. Additionally, participants should not have received an H5 influenza vaccine at any point. Example DMID studies include Protocol Nos. 05-0127, 07-0022, 08-0013, and 15-0066.

Subjects who use high-dose inhaled or nebulized corticosteroids or who have taken oral corticosteroids, or been administered parenteral corticosteroids during the 30 days prior to study receipt of study product will be excluded.

Subjects who have immunosuppression as result of treatment including a recent history (within 6 months prior to administration of study vaccine) or use of immunosuppressive or other immune-modifying drugs will be excluded. Low dose topical and intranasal steroid preparations are permitted.

Any anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to receipt of study product are not permitted.

Subjects should not have received blood or blood products (except Rho D immunoglobulin) during the 90 days prior to dosing.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Halting Criteria and Discontinuation of Study Intervention

7.1.1 Study Halting Criteria

The study halting criteria applies to the Low Dose, Medium Dose, High Dose, Optimal Dose Groups, but does not apply to the IIV4 Comparator Group. The Optimal Dose Group will be included in the respective dosing group (i.e., Low Dose, Medium Dose, or High Dose).

Additional enrollment and study interventions/administration of the study product in this trial will be halted for SMC review/recommendation if any of the following are reported:

- One or more subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration during the duration of the study.
- One or more subject with probable or confirmed myocarditis, pericarditis, or myopericarditis during the duration of the study.
- One or more subject presents with laryngospasm, bronchospasm, hypotension or anaphylaxis occurring within 24 hours following product administration that is considered related to study product.
- Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product.
- One or more subject experiences an SAE within 30 days of receipt of study product that is considered related to study product.
- Two or more subjects who received study vaccine to date across all dosing groups experience the same severe Grade 3 (unsolicited AE or safety laboratory), in the same High Level Term (HLT) by MedDRA coding other than myocarditis, pericarditis or myopericarditis, considered to be related to study product.

This trial will also be halted for SMC review/recommendation if, within 7 days after administration of study vaccination, any of the following occurs:

- Two or more subjects who received study vaccine to date within a dosing group experience the same severe (Grade 3) study vaccine-related injection site reaction within 7 days post-vaccination. Erythema and induration (hardness) will also be measured in mm but size will not be used as halting criteria.
- Two or more subjects who received study vaccine to date within a dosing group experience the same severe (Grade 3) study vaccine-related subjective systemic reaction within 7 days post-vaccination.
- Two or more subjects who received study vaccine to date within a dosing group experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction within 7 days post-vaccination.

Grading scales for solicited injection site and systemic (subjective and quantitative reactions) and clinical safety laboratory AEs are included in **Appendix A**.

If any of the halting rules are met following any subject receipt of any study vaccination, then this trial will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the SMC to proceed.

7.1.2 Individual Halting Criteria

Not applicable

7.1.3 Criteria for redosing

Not applicable

7.1.4 Follow up for Subjects Who Discontinued Study Intervention

Not applicable

7.1.5 Follow up for Subjects with a Suspicion for Myocarditis and/or Pericarditis

In the event that a subject develops symptoms, which in the judgement of investigator are consistent with a case of suspected myocarditis and/or pericarditis, the site will conduct an unscheduled telephone, video or in-person visit. The site will coordinate an appropriate diagnostic workup to make a determination of probable or confirmed myocarditis and/or pericarditis. The site will obtain and review the participants medical records from the diagnostic workup. The suspected myocarditis and/or pericarditis case should be reported to the DMID Pharmacovigilance Group within 24 hours of site awareness.

The definitions in **Appendix B** are intended to service as a guide to help in the reporting of suspected cases of myocarditis and/or pericarditis; however, the diagnosis of suspected cases is left to the investigator's clinical judgement.

7.2 Participant Withdrawal from the Study and Replacement

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Subject becomes pregnant prior to study product dosing. See **Section 8.4.11**
- Study non-compliance that in the opinion of the investigator poses an increased risk (i.e., missing safety labs), or compromises the validity of the data.
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- If the participant met an exclusion criterion for participation in the study (either newly developed or not previously recognized) that precludes further study participation.
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses.
- Subject lost to follow-up.

If the subject agrees, every attempt will be made to follow all AEs through resolution.

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and after administration of the study product will not be replaced. Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced.

The reason for participant discontinuation or withdrawal from the study will be recorded on a Case Report Form (CRF).

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

Screening procedures may be performed up to 14 days prior to enrollment (Day-14 to Day -1). After informed consent the following assessments are performed to determine eligibility and obtain baseline data:

- Collection of demographic data
- Take a focused medical history, including the following information:
    - History of any chronic medical conditions.
    - History of any medication allergies including history of hypersensitivity to any component of the study products.
    - Concomitant medications taken within 90 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility.
    - Ask if they are participating in another clinical trial or plan to enroll in another clinical trial during the study period.
    - Obtain history of influenza vaccination for the 2021-2022, 2022-2023, and 2023-2024 influenza seasons.
- Women of childbearing potential should be counseled to either practice true abstinence or use at least one primary form of contraception from 30 days prior to receipt of study product until 30 days after receipt of the study vaccine.
- Sexually active heterosexual men should be counseled to refrain from sperm donation and abstain from sexual intercourse or use contraception from the date of receipt of the study vaccine until 60 days after receipt of the study vaccine. (This does not apply to men in the immunogenicity comparator group receiving seasonal influenza vaccine)
- Women of childbearing potential must have a negative serum HCG pregnancy test at screening and a negative urine HCG pregnancy test within 24 hours of administration of study product.

- A physical examination will be performed on all subjects, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, to include general appearance and the following organs, organ systems and/or body areas: abdomen, heart, extremities, head, eyes, ears, nose, and throat, lymph nodes, musculoskeletal, neck, neurological, lungs, chest and skin. Height and weight measurement (Calculation of BMI).
- Vital signs measurement (including heart rate, blood pressure, oral temperature).
- Blood for laboratory evaluations.
- Serology at screening: HIV 1 and 2 antigen/antibody, Hepatitis B surface antigen, Hepatitis C antibody.
- Hematology studies at screening: WBC with differential, hemoglobin, and platelet count.
- Coagulation studies at screening: PT, APTT, and fibrinogen.
- Chemistry studies at screening: creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (Tbili), lipase, total iron binding capacity (TIBC), serum iron, and ferritin.
- Troponin (hsTnI).
- Urine Toxicology Screen for non-prescribed amphetamines, cocaine, and opiates.
- 12-lead ECG

Clinical screening laboratory evaluations will be performed locally by the Duke University Health System Clinical Laboratories. Urine pregnancy tests will be performed by research staff. The volume of venous blood to be collected is presented in **Section 4.1 Table 3.**

The overall eligibility of the subject to participate in the study will be assessed when all screening values are available. Study subjects who qualify will be entered into the Advantage eClinical system and all others will be registered as screen failures. Results of screening testing will be discussed with the subject. If a positive result for HIV, hepatitis C or hepatitis B occurs or there is a laboratory result deemed to be clinically significant by the PI, the subject will be referred for appropriate follow-up. Laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to laboratory error may be repeated once. An ECG that is determined to be clinically significant will not be repeated.

8.1.1 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

If a physiologic parameter, (e.g., vital signs) is outside of the protocol-specified range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is the result of an acute, short-term, rapidly reversible condition (e.g., stress, anxiety, or “white coat syndrome”). A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by malfunctioning, or an inappropriate measuring device (i.e., inappropriate-sized blood pressure cuff).

A subject may be re-screened if there is a transient disease status (e.g., subject complained of a "cold or fever" and met a temporary delaying enrollment criterion of acute illness or fever), or if a protocol eligibility criterion that is not met at the initial time of screening, will be met by rescreening at a later date (e.g., a medication taken within exclusionary window at the time of first screening that would not be within exclusionary window at a later rescreen).

Results of screening laboratory testing will be discussed with the subject. Participants will be provided the results of any abnormal clinical laboratory results, and will be referred to their primary healthcare provider. Screening laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to laboratory error may be repeated once. Research laboratory results will not be provided to the participant.

No subject may be screened more than twice due to a screening failure result as defined above.

8.2 Immunogenicity Assessments

8.2.1 Immunogenicity Evaluations

- Measurement of neutralizing and anti-HA stalk specific serum antibodies against the homologous H1N1 influenza strain at Days 1, 8, 15, 29, and 57

Please see **Section 4.1 Table 3** for venipuncture volumes.

8.2.2 Exploratory Assessments

- Anti-ferritin antibody determination (anti-human and *H. pylori* ferritin) at Days 1, 29, and 181
- Measurement of neutralizing and anti-HA stalk specific serum antibodies against the homologous H1N1 influenza strain at Days 85, 181, 366
- Neutralizing and anti-stalk serum antibody response to divergent H1 influenza virus isolates and other group 1 non-H1 influenza virus isolates at Days 1 and 29
- B cell and T cell, and T-follicular helper cell assays at selected time points in the entire study population or subsets of participants)

8.3 Safety and Other Assessments

Study procedures are specified in the **Schedule of Activities** table. A study clinician (Medical Doctor [MD]; Doctor of Osteopathy [DO]; Physician's Assistant [PA]; or Nurse Practitioner [NP]) licensed to make medical diagnoses and listed on the 1572 will be responsible for all trial-related medical decisions. Specimens for clinical screening and safety laboratory evaluations will be transported from the participating clinic sites to the respective site's certified clinical laboratories or on-site certified laboratories for analysis.

Ongoing Abstinence and Contraceptive Counseling

- Women of childbearing potential should be counseled to either practice true abstinence or use at least one primary form of contraception until 30 days after receipt of the study vaccine.
- Sexually active men should be counseled to refrain from sperm donation and abstain from sexual intercourse or use contraception until 60 days after receipt of the study

vaccine. (This does not apply to men in the immunogenicity comparator group receiving seasonal influenza vaccine)

Physical Examination

A physical exam will be conducted at screening (Day -14 to -1) by a licensed study clinician. A targeted physical exam will be performed at in-person visits (Days 1, 3, 8, 15, 29, 57, 85, 181 and 366) at the investigator's discretion.

Vital Signs

Vital signs will be assessed at screening (Day -14 to -1) and at in-person visits (Days 1, 3, 8, 15, and 29). Vital signs will include pulse, systolic blood pressure, diastolic blood pressure, and oral temperature. Vital signs may be collected at the investigator's discretion at Days 57, 85, 181, and 366.

Electrocardiograms (ECGs)

A 12-lead ECG will be performed at screening (Day -14 to -1), and Day 3, and as clinically indicated and confirmed by the PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572. Screening ECGs must be deemed not clinically significant.

Clinical Laboratory Evaluations

- Serology at screening: HIV 1 and 2 antibody, Hepatitis B surface antigen, Hepatitis C antibody
- Hematology (at screening and Study Days 1, 8, 29, 57 and any unscheduled visit or early termination visit at the investigator's discretion): white blood cells (WBC) with differential, hemoglobin, and platelets
- Coagulation studies (at screening and Study Days 1, 8, 29, 57 and any unscheduled visit or early termination visit at the investigator's discretion): PT, APTT, and fibrinogen.
- Chemistry studies (at screening and Study Days 1, 8, 29, 57 and any unscheduled visit or early termination visit at the investigator's discretion): creatinine, ALT, AST, ALP, Tbili, lipase, TIBC, serum iron, and ferritin
- Troponin will be tested at screening, and as clinically indicated and confirmed by the PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 will be collected, and tested at the discretion of the PI.
- Serum HCG pregnancy test at screening
- Urine HCG pregnancy test within 24 hours of receipt of study product on Study Day 1
- Urine toxicology test at screening

8.3.1 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 is responsible for recording all AE/SAEs that are observed or reported during this study, regardless of relationship to the study product. AE/SAEs, abnormal laboratory test values or abnormal clinical findings will be collected, assessed, documented, reported, and followed

appropriately, using a local laboratory as necessary. In determining eligibility, refer to **Section 5.1** and **5.2**, and the protocol-specific MOP.

8.3.2 Unscheduled Visit

If the investigator deems the reaction warrants further evaluation or intervention, the investigator will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation at an unscheduled visit if appropriate.

8.3.3 Early Termination Visit

Should the participant withdraw or be withdrawn or terminated from this trial, the study team will request a final in-person visit for an evaluation, to include a review of changes in medical history (including influenza vaccination history), concomitant medications, review for potential AEs (including MAAEs, NOCMCs, AESIs, SAEs) and possible targeted physical examination, ECG, vital signs, and laboratory evaluations at the discretion of the investigator. Depending upon the time of the early termination visit the participant will receive abstinence and contraceptive counseling.

8.3.4 Influenza-like Illness Visits

During the study, participants will be requested to report if they have any influenza-like illness (ILI) starting from the day of enrollment through Day 366. Subjects will be given instructions to notify the research team immediately with symptoms of ILI at each encounter with the research team and through a weekly electronic memory aid check beginning on Day 7. If subjects elect to receive reminders, they will be prompted through email messages to complete the electronic memory aid check within +/- 2 days of the weekly target.

Participants reporting influenza or ILI will be evaluated. ILI is defined as fever (temperature of 100°F [37.8°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 reverse transcription polymerase chain reaction (RT-PCR) in subjects who meet criteria for ILI. The severity of illness in subjects with laboratory confirmed influenza illness will be recorded through the AE form.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An AE can therefore be any unfavorable and unintended sign (including an increase in grade in an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. Clinical laboratory AEs are defined as laboratory abnormalities with a grade level increase in clinical laboratory values (as graded in **Appendix A**).

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

Adverse events can be further divided into solicited AEs and unsolicited AEs. Solicited AEs are those for which the study team will specifically query the participant whether they occurred.

Unsolicited AEs are those events that the subject reports occurring without being queried about the specific event.

All AEs will be assessed for severity and relationship to study intervention (see **section 8.3.3**).

All AEs, solicited and unsolicited, will be captured on the appropriate data collection form. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship.

AEs will be followed through resolution.

8.4.1.1 Solicited Adverse Events

Solicited AEs will be collected through an electronic memory aid in Advantage eClinical^{SM} (Electronic Data Capture System) or through a paper memory aid and are anticipated local and systemic AEs for which consistent collection of information is desired. This may include:

- Common and expected events according to the available knowledge about the product, or
- Specific events of concern that should be ascertained on every participant.
- Solicited AEs as noted in **Appendix A**, Local and Systemic Tables for Clinical Abnormalities (A and B), as well as temperature will be collected from each participant on Days 1-14 via the memory aid (eDiary).

8.4.1.2 Unsolicited Events

All AEs spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures must be recorded in the source document. All reported unsolicited AEs are graded in accordance with the protocol toxicity tables.

8.4.1.3 Special Reporting of Adverse Events

Not applicable

8.4.2 Definition of Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes (21 CFR 312.32 (a)):

- 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, convulsions that do not result in inpatient hospitalization, etc.

All SAEs, as with any AE, will be assessed for severity and relationship to study intervention (see **section 8.3.3**).

All SAEs will be recorded on the appropriate SAE data collection form and eCRF.

All SAEs will be followed through resolution by a licensed study clinician (for IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or sub-investigator).

All SAEs will be reviewed and evaluated by DMID and will be sent to the SMC (periodic review unless related), and the IRB/IEC.

8.4.2.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is any SAE where a causal relationship with the study product is at least reasonably possible but is not listed in the Investigator Brochure, Package Insert, and/or Summary of Product Characteristics.

8.4.3 Classification of an Adverse Event

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

8.4.3.1 Severity of Event

All clinical and laboratory AEs or SAEs will be assessed for severity, according to the FDA Guidance for Industry "Toxicity Grading Scales for healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007). (**Appendix A**). Note: Abnormal results as determined by the institutional laboratory for iron, total iron binding capacity and ferritin will not be assessed for severity according to toxicity tables.

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention. The event generally does not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection

form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

8.4.3.2 Relationship to Study Intervention

For each reported adverse reaction, the PI or designee must assess the relationship of the event to the study product using the following guideline:

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

For this study:

- Solicited AEs will be collected from Study Day 1, through 14 days after study vaccination and will be reviewed at Visit 4, Day 15.
- Non-serious unsolicited AEs will be collected from Study Day 1, through 28 days after study vaccination and will be reviewed at Visit 5, Day 29.
- Clinical laboratory AEs will be collected from Study Day 1, through 28 days after study vaccination.
- SAEs will be collected from the Study Day 1, through the end of the study (Day 366)
- Adverse events of special interest (AESI) corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, in addition to MAAEs and NOCMCs as defined in **Section 8.4.8** will be collected from Study Day 1, through the end of the study (Day 366). AESIs corresponding to myocarditis, pericarditis, or myopericarditis will be collected from Study Day 1, through 57 days after study vaccination.

8.4.5 Adverse Event Reporting

8.4.5.1 Investigators Reporting of AEs

Information on all AEs should be recorded on the eCRF. All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality. Each AE will also be described in terms of duration (start and stop date), severity, association with the study product, action(s) taken, and outcome.

8.4.6 Serious Adverse Event Reporting

8.4.6.1 Investigators Reporting of SAEs

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group.

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USASAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the Advantage eClinical[SM] (EDC System). Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to the study product, the site PI or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

8.4.6.2 Regulatory Reporting of SAEs

Following notification from the site PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any SUSAR in an IND safety report to the FDA and will notify all participating site PIs (i.e., all PIs to whom the sponsor is providing drug under its IND(s) or under any PIs IND(s) of potential serious risks from clinical studies or any other source, as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the Agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

SAEs that are not SUSARs will be reported to the FDA at least annually in a summary format which includes all SAEs.

For US-licensed vaccines such as IIV4: Established in 1990, the Vaccine Adverse Event Reporting System (VAERS) is a national early warning system to detect possible safety problems in US-licensed vaccines. VAERS is co-managed by the CDC and the FDA. VAERS accepts and analyzes reports of AEs (possible side effects) after a person has received a vaccination. Anyone can report an AE to VAERS. Healthcare professionals are required to report certain AEs and vaccine manufacturers are required to report all AEs that come to their attention.

VAERS is a passive reporting system, meaning it relies on individuals to send in reports of their experiences to CDC and FDA. VAERS is not designed to determine if a vaccine caused a health problem, but is especially useful for detecting unusual or unexpected patterns of AE reporting that might indicate a possible safety problem with a vaccine. This way, VAERS can provide

CDC and FDA with valuable information that additional work and evaluation is necessary to further assess a possible safety concern.

Link to VAERS https://vaers.hhs.gov/.

8.4.7 Reporting Events to Participants

Not applicable

8.4.8 Adverse Events of Special Interest

Adverse Events of Special Interest (AESIs) are a pre-defined subset of AEs that may be required to be collected at the request of the regulatory agency, industry partner or DMID, and is meant to address a specific safety concern. Any AESI that meets serious criteria must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group.

For the purpose of this study, AESIs are the following: myocarditis, pericarditis, or myopericarditis occurring following receipt of study vaccine through Day 57. Cases of probable and confirmed myocarditis, pericarditis or myopericarditis will be defined using the published CDC case definition (**Appendix B**). [16]

Additionally, any Grade 2 or greater decrease in lymphocyte or neutrophil count occurring following receipt of study vaccine through the entire study period will be classified as an AESI.

All AESIs are assessed, recorded, and followed as described above under AE. In addition, the AESI will be reported on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

8.4.9 Medically-Attended Adverse Events

For each unsolicited AE experienced, the subject will be asked if he/she had received medical attention, defined as hospitalization, an emergency room visit or an otherwise unscheduled visit to or from medical personnel for any reason. AEs characterized by such unscheduled medical care will be designated as MAAEs and will be reported in the Advantage eClinical℠ (EDC System).

8.4.10 New-Onset Chronic Medical Conditions

New-Onset Chronic Medical Conditions are defined as any new ICD-10 diagnosis (10th revision of the International Statistical Classification of Diseases and Related Health Problems) that is applied to the subject during the course of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention. NOCMCs will be reported in the Advantage eClinical℠ (EDC System).

8.4.11 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage eClinical℠ (EDC System) on the Pregnancy Report form. If the pregnant subject is agreeable, venous blood samples for serological assays will still be collected per protocol; however, large volume blood samples for cellular immunological assays will be discontinued. For male subjects with a pregnant partner, the pregnant partner will be asked to sign a Pregnant Partner Information Form to consent to collection of health information related to the pregnancy. If additional consent is not provided by the pregnant partner, the pregnancy outcome and date may be obtained from the research participant. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

The Department of Health and Human Services OHRP considers unanticipated problems (UP) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- 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 participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the reviewing IRB, the Statistical and Data Management Coordinating Center *(*SDMCC), and the lead PI. The UP report will include the following information:

- Protocol identifying information including protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the SDMCC/study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the SDMCC/study sponsor within three days of the investigator becoming aware of the problem.

8.5.3 Reporting Unanticipated Problems to Participants

Not applicable

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

This study is not designed to achieve pre-determined levels of power or precision to address the primary, secondary, or exploratory objectives. The power and precision allowed by the study's sample size are discussed in **Section 9.2**, and the general methodology planned to address study objectives is described in **Section 3**. No formal hypothesis testing is planned for primary and secondary objectives. Any potential hypothesis testing will be considered exploratory and will be detailed in a separate statistical analysis plan (SAP). Details about planned tables, figures, and data listings will be presented in a separate SAP.

9.2 Sample Size Determination

The primary objective of this study is to determine the safety of the VRC H1ssF_3928 mRNA-LNP vaccine. This is assessed by the incidence of solicited reactogenicity events, unsolicited AEs, MAAEs, NOCMCs, AESIs, ILIs, SAEs, and clinical laboratory AEs after vaccination. **Table 5** gives the probability of observing one or more safety events, such as a reactogenicity event or an AE of a particular classification, for varying sample sizes, given underlying true event probabilities.

Table 5: Probability (%) of observing at least one safety event, given varying underlying event probabilities and sample sizes

True Event Probability	Individual Cohorts		Optimal Dose Cohort
	Sentinel Lead-In N=2	Sentinel + Expanded Cohort N=10	N=20
0.01% (Very rare)	<0.1	0.1	0.2
0.1% (Rare)	0.2	1	2
1% (Uncommon)	2	9.6	18.2
10% (Common)	19	65.1	87.8
30% (Very common)	51	97.2	99.9

Among the 2 subjects in each of the cohorts considered for the safety reviews prior to enrolling the expanded cohort, there will be a 2.0% chance of observing at least one uncommon event and a 19% chance of observing at least one common event. When assessing the sentinel + expanded

cohort (N=10) as well as the optimal dose cohort (N=20), there will be a 9.6% and a 18.2% chance of observing at least one uncommon event, respectively, and a 65.1% and an 87.8% chance of observing at least one common event, respectively.

While the study was not designed to achieve a pre-determined level of precision, confidence intervals will be estimated for some of the binary endpoints. **Table 6** presents the exact 95% Clopper-Pearson confidence intervals that would result from observing varying numbers of events in groups of possible interest.

Table 6: Estimated event probabilities (%) and associated exact 95% Clopper-Pearson confidence intervals resulting from varying numbers of events observed in groups of different sizes.

Number of Observed Events	Individual Cohorts		
	Sentinel Lead-In N=2	**Sentinel + Expanded Cohort N=10**	**Optimal Dose Cohort N=20**
0	0 (0, 84.2)	0 (0, 30.8)	0 (0, 16.8)
1	**50 (1.3, 98.7)**	10 (0.3, 44.5)	5 (0.1, 24.9)
2	100 (15.8, 100)	20 (2.5, 55.6)	10 (1.2, 31.7)
5	-	**50 (18.7, 81.3)**	25 (8.7, 49.1)
6	-	60 (26.2, 87.8)	30 (11.9, 54.3)
10	-	100 (69.2, 100)	**50 (27.2, 72.8)**
12	-	-	60 (36.1, 80.9)
16	-	-	80 (56.3, 94.3)
20	-	-	100 (83.2, 100)

- For illustration, if zero events of a certain type were observed in an individual sentinel lead-in group with N=2 subjects, true event probabilities above 84.2% could be ruled out at the 95% confidence level, compared to 30.8% in a sentinel + expanded cohort (N=10) and 16.8% in the optimal dose group (N=20). Binomial confidence intervals are widest at 50% events observed; the estimates associated with 50% observed events are bolded in **Table 6.** Within a sentinel lead in group with N=2 subjects, the maximum half-width for a 95% CI would be 48.7%, compared to 31.3% in a sentinel + expanded cohort (N=10) and 22.8% in the optimal dose group (N=20). In the case of substantial loss to follow-up or missed visits, these estimates give an idea of the loss of precision due to decreasing the effective sample size.

9.3 Populations for Analyses

- The Safety population will include all subjects who receive the study vaccination.
- The Intent-to-Treat (ITT) population will consist of all subjects who received the study vaccination.

- The Per-Protocol (PP) population will include subjects in the ITT population with the following exclusions of data:
    - Data from participants determined to be ineligible at baseline
    - Data from any visit that occurs substantially out of window as defined in the SAP
    - Data from all visits subsequent to major protocol deviations, as defined in the SAP, that could impact the validity of later data, such as:
        - Receipt of immunosuppression or any medications that may be associated with impaired responsiveness
        - Receipt of any non-study investigational drug/investigational vaccine/licensed vaccine as defined in the exclusion criteria

The Safety population will be used for all analyses of AEs and reactogenicity. Immunogenicity analyses will be done in the ITT population. The PP population will be used for sensitivity analyses if more than 10% of secondary analysis observations across Days 8, 15, 29, and 57 are excluded by the PP exclusions.

9.4 Statistical Analyses

The primary clinical database for this study will consist of safety data including solicited reactogenicity events, unsolicited events, and abnormal laboratory values, as well as baseline/demographic data. Once the last participant has completed the final visit, the primary clinical database will be cleaned, monitored, and locked. After clinical database lock and receipt of secondary endpoint immunogenicity data, a set of topline tables will be generated by the SDMCC, including summaries of clinical safety and secondary immunogenicity data. The topline report will be made available to the study team for planning subsequent trials and may be presented in a public forum or used for publication in collaboration with the lead PI. These analyses will be considered final and will be included in the Clinical Study Report (CSR). The CSR, comprised of the final analyses of safety and available immunological data, will be subsequently completed. The final CSR will be completed when all primary and secondary safety, clinical, and immunological endpoint data are available. Any available data from the exploratory endpoints at the time of compilation of the final CSR may also be included. Additional exploratory endpoint data may be included in an addendum to the CSR, publication of manuscript(s), or other report.

A formal SAP, which will elaborate on the analyses described here and describe any changes to the planned analyses, will be developed and finalized before performing any of these analyses (with the exception of SRC/SMC reviews of descriptive safety analyses).

In addition to the descriptive analyses of study data for SRC/SMC review, an interim analysis is planned for the ongoing study to allow for an early assessment of the immunogenicity of the study vaccine and to aid in decision-making regarding future studies. This analysis would not affect the conduct of this trial via early stopping. Additional details on interim analysis are given in **Section 9.4.6**.

9.4.1 General Approach

The non-missing sample size, mean, standard deviation, and the minimum, median, and maximum will be summarized as a descriptive analysis for all continuous variables. Categorical

variables will be descriptively summarized by frequencies and percentages of observed levels, based on the non-missing sample size. Titers will be summarized with geometric means, first across technical replicates to compute one value for each individual sample (where applicable) and then applied again across participants within the same group.

In general, summaries will be presented by analysis group whereby non-optimal dose cohorts will include the sentinel and expanded participants and the optimal dose cohort will include the sentinel and expanded as well as the participants assigned to the cohort after the dose was chosen as optimal. All tables will be annotated with the total population size relevant to that table, including any missing observations. The analysis population for each exhibit will be clearly indicated.

Exact Clopper-Pearson confidence intervals will be computed for binary variables, where applicable. The preferred approach for descriptively summarizing continuous variables will include confidence intervals computed based on the t-distribution with n-1 degrees of freedom, where n denotes the number of observations. If the data are found to be non-normal, and the sample size is insufficient for application of the central limit theorem, nonparametric bootstrap confidence intervals may be calculated instead.

Participants are able to receive the seasonal influenza vaccine more than 90 days before enrollment and after 60 days post-study vaccination. Therefore, in order to control for this potential confounding, immunologic endpoints assessed prior to Day 60 will be stratified (where possible) by prior season's receipt of the seasonal influenza vaccine within an analysis group and summarized, and immunologic endpoints assessed after Day 60 will be stratified (where possible) by prior season's and/or subsequent receipt of the seasonal influenza vaccine within an analysis group and summarized.

9.4.2 Safety Analysis

The primary objective of the study is to assess the safety of a single dose of the study vaccination, as determined by the incidence of solicited reactogenicity events, unsolicited serious and non-serious AEs, MAAEs, NOCMCs, AESIs, ILIs, SAEs, and clinical laboratory AEs.

Safety objectives will be assessed in the Safety population. All summaries will be provided by analysis group as described in **Section 9.4.1**. Solicited local and systemic reactogenicity events will be summarized via the number and percentage of participants reporting each event, and any solicited reactogenicity event, through 14 days post-vaccination. These will be tabulated by severity and any severity. Exact 95% Clopper-Pearson confidence intervals for the probability of each event occurring collectively across any severity level post-vaccination will be computed for each group. Severity will be determined as described in **Section 8.4.3.1**.

Non-serious unsolicited AEs through 28 days post-vaccination will be summarized by MedDRA system organ class (SOC), MedDRA preferred term and severity. AESIs corresponding to myocarditis, pericarditis, or myopericarditis reported through Day 57 and AESIs corresponding to Grade 2 or greater decrease in lymphocyte or neutrophil count, MAAEs, NOCMCs, ILIs, and SAEs reported through the end of the study period (one year post-vaccination) will be summarized similarly. Clinical laboratory AEs are defined as a grade level increase in clinical laboratory values (as graded in **Appendix A**) and will be summarized separately. For unsolicited

events, summaries regarding relationship to study vaccine will also be provided, as described in **Section 8.4.3.2**.

AEs and abnormal clinical laboratory results, including clinical laboratory AEs, will be presented in data listings.

9.4.3 Secondary Immunogenicity Analysis

The secondary objective of the study is to assess the serum antibody response of a single dose of the study vaccine as compared to a standard dose of IIV4. This response will be assessed via homologous H1-specific neutralizing antibodies in a reporter microneutralization assay, homologous H1-specific neutralizing antibodies in a viral microneutralization assay, and anti-HA stalk serum antibodies. Assays will be analyzed separately by analysis cohort as described in **Section 9.4.1**.

The GMT and corresponding 95% confidence intervals will be presented for applicable endpoints by analysis cohort at Days 1, 8, 15, 29, and 57. For post-baseline time points (i.e., Days 8, 15, 27, and 57), the GMFR from baseline and corresponding 95% confidence intervals will be presented by analysis group. Summaries stratified by prior season's receipt of the seasonal influenza vaccine within an analysis group will also be presented.

9.4.4 Exploratory Immunogenicity Analysis

The exploratory objectives of the study involve further characterization of the immunogenicity of the study vaccine and the seasonal IIV4 vaccine given to each participant after the vaccine has been administered, including anti-ferritin antibody response, durability of the immune response, antibody breadth, and B cell and/or T cell responses. Exploratory analyses of antibody responses will be assessed utilizing the same assays as those employed for secondary immunogenicity analysis, at additional timepoints and against divergent H1 or group 1 non-H1 influenza viruses.

Immune responses will be described by time point, analysis group, and stratified by prior season's receipt and subsequent receipt (where applicable) of the seasonal influenza vaccine within an analysis group, similarly to the summaries of secondary immunological endpoints. For assay data generated by serial dilutions, GMTs (ID_{50}) and GMFR will be calculated, where applicable, along with corresponding 95% confidence intervals. Other immunological endpoints will be described by means and associated 95% confidence intervals (bootstrapped if necessary) and/or medians and associated interquartile ranges (25^{th} percentile, 75^{th} percentile) depending on distributional properties.

Exploratory analyses may include statistical modeling of appropriately transformed endpoints over time while accounting for prior and subsequent vaccination status. These analyses will be described in the SAP, along with further details for analyses of exploratory immunogenicity endpoints and exploratory analyses of secondary immunogenicity endpoints.

9.4.5 Baseline Descriptive Statistics

Baseline and demographic characteristics will be summarized by analysis group, with summary statistics as described in **Section 9.4.1** for all enrolled participants. These characteristics will include age, sex, ethnicity, race, and prior season's receipt of seasonal vaccination.

9.4.6 Planned Interim Analyses

9.4.6.1 Interim Safety Analyses

There are two safety committees that will be convened for this study: The SRC and the SMC. These are described in detail in **Section 10.1.6**.

The SRC will review safety data after the first 2 participants in each active vaccine cohort (excluding the dose group after it has been chosen to be optimal) have completed 3 days of post-vaccination safety data to determine whether the remaining eight participants in the cohort may begin enrollment, and again after all 10 participants in each active vaccine cohort (excluding the dose group after it has been chosen to be optimal) have completed 7 days of post-vaccination safety data to determine whether the next highest dosing cohort may begin enrollment. The SMC will review safety data if deemed necessary by the SRC prior to enrolling the expanded portion of a cohort, if a halting rule (as defined in **Section 7.1**) occurs, or as regularly scheduled per the separate SMC charter.

Interim safety reviews may include enrollment and demographic information, clinical laboratory tests, and AE/SAEs. During SMC reviews, additional data may be requested by the SMC. As an outcome of each SMC review, the SMC will make a recommendation to continue, modify, or terminate this study.

9.4.6.2 Interim Immunogenicity Review

As described in **Section 9.4**, an interim analysis focused primarily on immunogenicity will be conducted while the study is ongoing. Results of this analysis may be used for planning for future studies, with no public dissemination of results, and not used to make any decisions concerning the conduct of this trial. Interim immunogenicity analyses of humoral responses through Day 57 will be presented in aggregate by cohort. Additional details regarding the interim analysis will be provided in a memorandum to DMID and the PI prior to generating the report.

9.4.7 Sub-Group Analyses

There are no planned sub-group analyses for this study due to small sample sizes. Stratified analyses will be performed to account for potential confounding factors (e.g., prior or subsequent receipt of a seasonal vaccine) in applicable analyses as described in **Sections 9.4.3** and **9.4.4**.

9.4.8 Tabulation of Individual Participant Data

Safety data, reactogenicity data, and immunogenicity data will be presented in listings, as described in **Section 9.4.2**.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

Each institution engaged in this research will hold a current Federal Wide Assurance (FWA) issued by the OHRP for federally funded research and provide the FWA number to DMID.

The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6(R2) GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human

Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

10.1.1 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The key information about the purpose of the study, the procedures and experimental aspects of the study, study interventions/products, probability for random assignment to treatment groups, risks and discomforts, the expected duration of the subject's participation in the trial, any expected benefits to the subject, and alternative treatments and procedures that may be available to the subject. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.

Subjects will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the ICF prior to starting any study procedures for this trial. Once signed, a copy of the ICF will be given to the subject(s) for their records.

New information will be communicated by the site PI to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated, and subjects will be re-consented per IRB requirements, if necessary.

10.1.1.1 Other Informed Consent Procedures

The rights and privacy of human subjects who participate in genomic or phenotypic research studies will be protected at all times. No genomic testing is planned for this study; however, the subject will be asked to consent for future use of specimens for secondary research; genetic testing on these samples is likely to be performed. Please refer to **Section 10.1.4.1**. The consent process, including relevant language in the ICF, will provide an explanation of the potential risks to the individual study subjects and their families. The consent will include whether individual subject data will be shared through a NIAID-designated controlled access data repository. Clinical metadata, genomic, or other datasets or a subset of the clinical and other metadata that may potentially identify human subjects will not be released in unrestricted databases. Subjects will be informed that the evolution of genomic technology and analytical methods raises the risk of re-identification, even when specimens are de-identified.

In addition to this protocol, subjects will be asked to consent to a separate biorepository protocol for the use of residual samples and associated data in future research. The extra samples collected for the repository are noted in this study's consent form and in this protocol. The subjects will be informed that if they do not agree with the collection of the extra samples for research, they should not enroll in this study. Subjects will be informed that they may withdraw their consent for the collection and use of the extra samples at any time without any penalty. If they withdraw consent, they will continue in the study and will not have the additional blood collected. If the subject wants the collected specimens destroyed, only the unused specimens in the repository will be destroyed.

10.1.2 Study Termination and Closure

Section 7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal, describes the temporary halting of the study.

This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Halting criteria met and it is deemed unsafe to proceed with further dosing
- Insufficient compliance with protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Decision by regulatory authorities

If the study is prematurely terminated, the PI will promptly inform study participants, the IRB and regulatory authorities as applicable. Study participants will be contacted, as applicable, and

be informed of changes to the study visit schedule. The PI will assure appropriate follow-up for the subjects, as necessary.

The sponsor will notify regulatory authorities as applicable.

10.1.3 Confidentiality and Privacy

Subject confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to participants, test results of biological samples and genetic tests, and all other information generated during participation in the study. No identifiable information concerning participants in the study will be released to any unauthorized third party. When a participant's hepatitis B, hepatitis C, or HIV screening result is positive, the result will be reported to the North Carolina Division of Public Health. The test results will be kept confidential to the extent permissible under the law. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor, other authorized representatives of the sponsor, representatives of the 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.

All source records including electronic data will be stored in secured systems in accordance with institutional polices and federal regulations.

All study data and research specimens that leave the site (including electronic transmission of data) will be identified by a coded number that is linked to a subject through a code key maintained at the clinical site. The code key, including names or readily identifiable information is kept confidential and will not be transmitted off site.

As this research is funded by the NIH, it is covered by NIH policy which effectively issues the research a Certificate of Confidentiality. By this policy, researchers cannot be forced to disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains.

The Certificate cannot be used to resist a demand for information from personnel of the US Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the FDA.

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, or for the purposes of other research that the release is in compliance with applicable Federal regulations governing the protection of human subjects in research.

10.1.4 Future Use of Stored Specimens and Data

10.1.4.1 Samples for Secondary Research

Eligible participants will be asked to sign a separate consent form for a separate biorepository protocol (Pro00104290, DMID No. 19-0025), for the storage and use of leftover and extra samples and associated data for the purpose of future research. Some extra specimens will be collected specifically for the purposes of secondary research and submitted to a repository per DMID Protocol No. 19-0025 and consent form. Samples designated for secondary research use may be used in additional assessments that may include but are not limited to, evaluation of mucosal antibody responses, functional antibody assays, and evaluation of the innate immune response. These specimens might be used in new or different immunological laboratory tests, to provide information for the development of new vaccines or therapeutics, or for the study of influenza or other infections. Specimens will be coded. Any future testing laboratory will not have access to the code, and therefore will not be able to identify study participants. Genetic testing on these samples is likely to be performed. The specifics of this secondary research testing will be in a separate protocol and will be reviewed by an IRB.

Samples will not be sold for commercial profit. Although the results of any future research may be patentable or have commercial profit, subjects will have no legal or financial interest in any commercial development resulting from any future research.

Risks are associated with the additional volume of blood collected, such as anemia. Risks for loss of privacy and confidentiality are described below.

Hence, the following types of samples will be stored and used for secondary research:

- Residual biological specimens - Any leftover primary research sample after laboratory testing is completed per protocol and which the protocol explicitly states is allowed to be stored and used for secondary research, and for which the research subject gave specific consent.
- Repository research sample - Samples will be collected with the subject's consent in this protocol with the intent to store for additional research (i.e., samples collected beyond those needed for primary research including nasal absorption specimen, additional whole blood for isolation of peripheral blood mononuclear cells, and PAXgene tube) and will be used in future studies.

To participate in this study, subjects must agree to the storage of samples for secondary use under DMID Protocol No. 19-0025. Subjects will be consented prior to enrollment in the study by a separate consent form. Samples will be stored indefinitely at a DMID-designated storage facility. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. Secondary research with coded samples and data may occur; however, subject confidentiality will be maintained as described for this protocol. An IRB review of the secondary research using coded specimens is required.

Residual/Repository Research Samples, upon written request and approval from DMID and any approvals required by the site, may be shared for secondary research with investigators at the participating site, with researchers at other Collaborative Influenza Vaccine Innovation Centers (CIVICs) sites or other institutions, or company-designated research laboratories. The samples will not be sold or used directly for production of any commercial product. DMID will authorize shipment from the repository.

Reports from secondary research will not be kept in the subjects' health records or shared with subjects, unless required by law. Reports will not be sent to the specimen repository.

The subject's decision for secondary research can be changed at any time by notifying the study doctors or nurses in writing. If the subject changes his/her decision, the samples will be destroyed if the samples have not been used for research or released for a specific research project.

10.1.4.2 Data Sharing for Secondary Research

Data from this study may be used for secondary research. All of the individual participant data collected during the trial will be identified by a coded number that is linked to a subject through a code key maintained at the clinical site. The code key is kept confidential and will not be transmitted off site. All of the individual participant data collected during the trial will be made available after de-identification. The Protocol, SAP, and Analytic Code will also be made available. This data will be available immediately following publication, with no end date.

10.1.5 Key Roles and Study Governance

The study is sponsored by DMID. Decisions related to the study will be made by the protocol team, which includes representatives from the participating sites (PI), DMID (sponsor), and the SDMCC. The PI is listed on the cover page. Other study team members and roles are listed in the protocol-specific MOP.

10.1.6 Safety Oversight

This clinical study will utilize an SRC. The SRC consists of the site PI (or their delegate), representatives of the sponsor (at least the Medical Monitor and the Medical Officer), representatives of the product developer (at least the Medical Lead or delegate), and representatives of the SDMCC. The SRC will review the data required to allow progression from cohort to cohort and selection of optimal dose. These reviews occur as outlined in the protocol. Ad hoc reviews may also be called for by the investigators or sponsor. Decisions are dependent on the failure to meet halting criteria when the required follow-up data are available. The SRC deliberations may occur by teleconference, videoconference, or email. If the SRC determines that the study data have met the criteria necessary to continue from a sentinel to expanded cohort or to begin a subsequent cohort, the sponsor will notify the site of the determination. If the SRC or any of its members is unable to determine that the criteria necessary to continue to an expanded cohort or to begin a subsequent cohort have been met, or if halting rules have been met, the SMC will convene. The DMID Medical Monitor may request an SMC review at any time.

As noted above, this clinical study will utilize a SMC. The SMC is an independent group of at least 3 experts that monitors participant safety and advises DMID. SMC members will be separate and independent of study personnel participating in this trial and should not have

scientific, financial, or other conflicts of interest related to this trial. The SMC will consist of members with appropriate expertise to contribute to the interpretation of data from this trial. A quorum will consist of a simple majority.

The SMC will hold an organizational meeting prior to enrollment. At this meeting, the SMC will review the charter, protocol, ICF, and safety report template.

The SMC will conduct the following reviews:

1. Review of safety if a halting rule is met
2. Review of safety if the SRC requests a review
3. Review of safety, tolerability, and trial progress at the conclusion of the trial
4. Ad hoc review as needed when requested by the sponsor, investigators, or SMC

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review applicable data including, but not limited to, enrollment, demographics, dosing, data, laboratory data and safety data at scheduled time points during this trial as defined in the SMC charter. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study drug administration, and to continue, modify, or terminate this trial.

10.1.7 Clinical Monitoring

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 IRB/IEC approved protocol/amendment(s), ICH GCP E6(R2), and applicable regulatory requirements. Clinical monitoring also verifies any critical study procedures were completed following specific instructions in ancillary documents referenced in the protocol such as the MOP.

Monitoring for this study will be performed by DMID. Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, how frequent the monitoring will be done, how detailed the monitoring will be, and when the monitoring reports will be distributed. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study staff and all study documentation according to the DMID-approved CMP. Study monitors will meet with all participating site PIs to discuss any problems and outstanding issues and will document site visit findings and discussions.

10.1.8 Quality Assurance and Quality Control

To ensure the reliability of study data, each site will develop a Clinical Quality Management Plan (CQMP). The CQMP will describe:

- Routine internal quality control (QC) and quality assurance (QA) activities

- For the purposes of measuring, documenting and reporting study conduct, protocol adherence, human subjects' protections, and reliability of the protocol-driven data collected;

- A process for addressing in a timely manner any data quality issues (i.e., collecting, recording and reporting data), systemic issues (i.e., protocol conduct, non-compliance, human subject protections), and implementation of Corrective and Preventive Actions (CAPA) procedures.

The SDMCC will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the SDMCC to the sites to record and maintain data for each subject enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Clinical research data (including, but not limited to, AEs/SAEs, concomitant medications, medical history, physical assessments), and clinical laboratory data will be abstracted from the source documentation and/or collected on the data collection form by study personnel then entered into eCRFs via the 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center Advantage eClinical℠ (EDC). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Data reported in the eCRF should be consistent with the data collection form/source documents or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Company, Inc., under subcontract to Digital Infuzion, Inc., will serve as the SDMCC for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

10.1.9.2 Study Record Retention

Study related records, including the regulatory file, study product accountability records, consent forms, subject source documents and electronic records should be maintained for a period of 2 years following the date a marketing application is approved for the investigational product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. These documents should be retained for a longer period, however, if required by local policies or regulations. No records will be destroyed without the written consent of DMID. Consent forms with specimen retention linked to identifiable specimens will be maintained for as long as the specimens remain in identifiable format, and a minimum of 3 years after use of the identifiable specimens in nonexempt human subject research. Site must contact DMID for authorization prior to the destruction of any study records.

10.1.9.3 Source Records

Source data are all information in original records (and certified copies of original records) of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data should be attributable, legible, contemporaneous, original, accurate, and complete (ALCOA-C). Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP E6(R2), regulatory, and institutional requirements. For this trial, hardcopies of the study visit worksheets will be provided for use as source document worksheets. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, any process that is noted in the protocol and refers to details in the protocol-specific MOP, or GCP requirements or any critical study procedures with specific instructions in ancillary documents referenced in the protocol such as a protocol-specific MOP.

The noncompliance may be either on the part of the subject, the investigator, or the study site staff. Following a deviation(s), corrective actions should be developed by the site and implemented promptly. All individual protocol deviations will be addressed in subject study records.

It is the responsibility of the site PI and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID via Advantage eClinical℠ (EDC). Protocol deviations must be sent to the IRB/IEC per their guidelines. The site PI and personnel are responsible for knowing and adhering to their IRB's requirements. All deviations from the protocol must be addressed in study data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart if the deviation is subject specific.

10.1.11 Publication and Data Sharing Policy

Following completion of the study, results of this research will be published in a scientific journal.

Data will be available immediately following publication, with no end date, with data sharing at the discretion of the Sponsor. Sites may also obtain individual or country level data from the database for separate publications is desired. Publication may occur prior to completion of a final CSR for the entire trial.

10.1.12 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.13 Publication

Following completion of the study, the lead PI is expected to publish the results of this research in a scientific journal. This study will adhere to the following publication and data sharing policies and regulations:

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH-funded research. As such, the final peer-reviewed journal manuscripts will be accessible to the public on PubMed Central no later than 12 months after publication.

10.1.14 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. DMID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Additional Considerations

10.2.1 Research Related Injuries

If it is determined by the site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this study, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided by the NIAID, NIH, or the federal government to the subject, for any injury suffered due to participation in this trial.

10.3 Abbreviations

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
APTT	Activated Partial Prothromboplastin Time
AR	Adverse Reaction
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BP	Blood Pressure
CAPA	Corrective and Preventative Actions
CDC	Centers for Disease Control and Prevention
cGMP	Current Good Manufacturing Practice
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CIVICs	Collaborative Influenza Vaccine Innovation Centers
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CMS	Clinical Material Services
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
dbGap	Database of Genotypes and Phenotypes
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DHVI	Duke Human Vaccine Institute
DGP	Duke Human Vaccine Institute GMP Program
DMID	Division of Microbiology and Infectious Diseases
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
ELISA	Enzyme Linked Immunosorbent Assay
ER	Emergency Room
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FWA	Federal Wide Assurance
GCP	Good Clinical Practice

Abbreviation	Definition
GLP	Good Laboratory Practices
GMFR	Geometric Mean Fold Rise
GMP	Good Manufacturing Practices
GMT	Geometric Mean Titer
GWAS	Genome-Wide Association Studies
H1ss	H1 Stabilized Stem
H1ssF_3928 mRNA Vaccine	VRC H1ssF_3928 mRNA-LNP drug product name
HAI	Hemagglutination Inhibition
HBV	Hepatitis B
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLT	High Level Term
HR	Heartrate
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IIV4	Inactivated Influenza Vaccine Quadrivalent
ILI	Influenza-Like Illness
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intention-To-Treat
LNP	Lipid Nanoparticle
LUC	Large Unstained Cells
MAAE	Medically-Attended Adverse Events
mcg	Microgram
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
mRNA	Messenger RNA
MSDS	Material Safety Data Sheet
N	Number (typically refers to subjects)
NA	Neuraminidase
NCT	National Clinical Trial
NOCMC	New-Onset Chronic Medical Conditions
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No-Adverse-Effect-Level
NP	Nasopharyngeal
OHRP	Office for Human Research Protections

Abbreviation	Definition
OHSR	Office for Human Subjects Research
PBMC	Peripheral Blood Mononuclear Cell
PEG	Polyethylene Glycol
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PII	Personally Identifiable Information
PP	Per-Protocol
PRN	As Needed
PT	Prothrombin Time
QA	Quality Assurance
QC	Quality Control
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDMCC	Statistical and Data Management Coordinating Center
SRC	Safety Review Committee
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
Tbili	Total Bilirubin
TIBC	Total Iron Binding Capacity
UP	Unanticipated Problem
US	United States
USP	US Pharmacopeia
VAERS	Vaccine Adverse Event Reporting System
VRC	Vaccine Research Center
WBC	White Blood Cells
WHO	World Health Organization

10.4 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
7.0	15 May 2024	Updated Section 9.4.6.1 to match the rest of the protocol that the SRC will review safety data after the first 2 participants in each active vaccine cohort have completed **3 days** of post-vaccination safety data, rather than 7 days	Administrative
6.0	26 Apr 2024	Added the 2023/24 flu season to data collected due to enrollment continuing past 2023/24 flu season	Administrative
		Added update of IB background information	Administrative
		Formatting updates	Administrative
5.0	24 Aug 2023	Appendix A: Hematology Table B Lymphocytes Decreased reference ranges updated to align with FDA toxicity ranges.	Administrative
4.0	25 Apr 2023	Sections 1, 3, 4, 8, 9 and SoA: Updated AESI's corresponding to myocarditis, pericarditis, or myocarditis will be collected from the time of vaccination through day 57.	Updated per SMC recommendation.
		Inclusion Criterion #7: updated pulse range to 50 to 100 beats per minute, inclusive.	Updated per medical monitor.
		Exclusion Criterion #4: removed cannabinoids from urine toxicology screen.	Updated per SMC recommendation.
		Table 3 Blood Volumes: footnote listing safety labs	Administrative

		Section 8.4.11 Reporting of Pregnancy: added pregnant partner will be asked to sign a Pregnant Partner Information form.	Updated per Duke IRB
		FDA toxicology table, Appendix A, Hematology: updated iron normal range for females.	Administrative Correction
3.0	20 Mar 2023	Removed Independent Safety Monitor (ISM) throughout	Administrative
		Troponin (cTnT) changed to Troponin (hsTnI) throughout. FDA toxicology table, Appendix A, Chemistry: Troponin (hsTnI) reference range updated.	Troponin will be processed by the Duke Lab rather than Labcorp. The type of troponin and reference ranges were updated.
		SoA footnote #15- The timeframe and mechanism for ILI reporting added.	Administrative
		SoA #18 updated for troponin. Removed that results will not be included in the participants EMR and that samples will be tested at PI discretion.	Troponin will be processed at Duke and the results will appear in the participants EMR.
		SoA footnote #21 updated to state that unscheduled visit may be conducted via phone or IRB-approved telehealth.	Administrative
		SoA footnote #21 and Section 8.3.4: ILI reporting period changed from screening through Day 366 to enrollment through Day 366.	Administrative
		Section 4.1: Language added to clarify when IIV4 comparator group can be enrolled.	Administrative
		Section 5.4: The time period after study vaccination in which	Administrative

		participants need to refrain from receiving an experimental agent or participating in another clinical trial with an interventional agent changed from 60 days to through the entire study period.	
		Section 6.2.1: Added study product sources.	Administrative
		Section 6.2.3: Updated to note that undiluted and diluted study product is stable at USP controlled temperature.	Administrative
		Section 7.1.1: Clarified that the study halting criteria applies to the Low Dose, Medium Dose, High Dose, Optimal Dose Groups and not the IIV4 Comparator Group.	Administrative
		Section 7.1.5, 8.4.8 and SoA: DMID Medical Monitor replaced with DMID Pharmacovigilance Group.	Administrative
		FDA toxicology table, Appendix A, Hematology: Increased Fibrinogen Grade 1 range updated.	Updated per Duke fibrinogen reference range.
		Section 3 Objectives and Endpoints table: footnote added	Updated per DMID ORA protocol template language.
		Section 9.4 Statistical Analysis: updated with revised template language	Updated per DMID ORA protocol template language.
		Table 3: Blood Volume Collection Summary Footnote	Updated language to allow for PI discretion when related to subject safety
2.0	17 Nov 2022	Section 4.1 and 8.4.4 updated to describe that solicited AEs will be reviewed at Visit 4, Day 15	Administrative

		and unsolicited AEs will be reviewed at Visit 5, Day 29.	
		Removed units (ID_{50} and ID_{80}) in secondary and exploratory endpoints.	Administrative
		Clarified inclusion criteria #11, exclusion criteria #14, #15, and #17.	Administrative
		Changed the order of exclusion criteria #24 and #25.	Administrative
		Section 2.3.1: Edited blood drawing risks.	Administrative
		Section 6.1.1: Added IIV4 description	Administrative
		Section 6.2.3: Added additional study product preparation instructions.	Administrative
		Section 6.5: Removed listing of immunosuppressive drugs	Administrative
		Section 8.4.8: Added reporting requirements when an AESI meets SAE criteria.	Administrative
		Section 8.4.11: Updated pregnancy consent and reporting.	Administrative
		Typographical and grammatical errors corrected throughout. Abbreviations and call outs updated throughout.	Administrative
		Added coagulation labs to exclusion criteria #3.	Administrative
		Added coagulation labs to schedule of events and sections 8.1, 8.3, to occur at screening, days 1, 8, 29, 57, unscheduled, and early termination visits.	Administrative

		Added specification to Schedule of Activities footnote #8, in regard to coagulation studies, to indicate that PI discretion should be used to determine whether additional blood draws should be obtained at subsequent study visits.	Administrative
		FDA toxicology table, Appendix B, Hematology, updated to include coagulation studies.	Administrative
		Deleted designation for the ionizable lipid and replaced with the chemical name. Deleted the designation for the PEG lipid.	Administrative
		Removed that the Immunogenicity Comparator subgroup will receive IIV4 during the seasonal influenza vaccine season.	To defer study start until after the 2022-2023 influenza season.
		Clarified eligibility criteria: exclusion criteria #24, and #25, as well as a typographical update to #26.	Administrative
		Added life style considerations pertaining to seasonal influenza vaccine in section 5.4	Administrative
		Changed the seasons for prior and subsequent influenza vaccine documentation	To defer study start until after the 2022-2023 influenza season.

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APPENDIX A

The FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007 are the basis for toxicity grading for the current trial. Some modifications have been made for the current trial and are noted in footnotes below each table.

Tables for Clinical Abnormalities

A. Local

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Pain	Does not interfere with activity	Use of non-narcotic pain reliever or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Erythema/Redness Functional*	Does not interfere with activity	Interferes with activity	Prevents daily activity
Induration/Hardness **	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration/Hardness Functional**	Does not interfere with activity	Interferes with activity	Prevents daily activity
Axillary lymph node swelling on same arm as injection	Does not interfere with activity	Interferes with activity	Prevents daily activity

* For each day measured, the greatest diameter of local reactions including erythema and induration should be recorded.

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

B. Systemic

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Oral Temperature (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	≥39.0 >102.0
Nausea	Does not interfere with activity	Interferes with activity	Prevents daily activity
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
Diarrhea	No interference with activity or 2 – 3 loose stools /24 hours	Some interference with activity or 4 – 5 stools/24 hours	Prevents daily activity, 6 or more watery stools or /24 hours or requires outpatient IV hydration
Headache	No interference with activity	Use of non-narcotic pain reliever or some interferencewith activity	Anyuse of narcotic pain reliever or prevents dailyactivity
Chills	No interference with activity	Some interferencewith activity	Prevents dailyactivity
Fatigue	No interference with activity	Some interferencewith activity	Prevents dailyactivity
Myalgia (muscle pain other than injection site)	No interference with activity	Some interferencewith activity	Significant; prevents daily activity
Arthralgia (joint ache)	No interference with activity	Some interferencewith activity	Significant; prevents daily activity

C. Vital Signs

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Oral Temperature (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	≥39.0 >102.0
Tachycardia - beats per minute	101 – 115	116 – 130	> 130
Bradycardia - beats per minute	50-54	45-49	< 45
Hypertension (systolic) -mm Hg	141 – 150	151 – 155	> 155
Hypertension (diastolic) -mm Hg	91 – 95	96 – 100	> 100
Hypotension (systolic) –mm Hg	85 – 89	80 – 84	< 80

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.

Table for Laboratory Abnormalities

A. Chemistry

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Creatinine – mg/dL± (Male) (Female)	1.4 – 1.7 1.1 - 1.5	1.8 – 2.0 1.6 - 2.0	>2.0 >2.0
Alanine aminotransferase (ALT) (increase by factor)	1.01 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Aspartate aminotransferase (AST) (increase by factor)	1.01 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Alkaline phosphatase (increase by factor)	1.01 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Bilirubin – when accompanied by any increase in AST or ALT (increase by factor)	1.01 – 1.25 x ULN	1.26 – 1.5 x ULN	> 1.5 x ULN
Bilirubin – when AST and ALT are normal; increase by factor	1.01 – 1.5 x ULN	1.6 – 2.0 x ULN	> 2.0 x ULN
Lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	> 2.0 x ULN
Troponin (hsTnI)	Severity will not be assessed. Normal range (Male ≤ 20 ng/L) Normal range (Female ≤ 14 ng/L)		
Iron	Severity will not be assessed. Normal range (Male 50 - 160 µg/dL) Normal range (Female 45- 150 µg/dL)		

Total Iron Binding Capacity	Severity will not be assessed. Normal range (261 - 478 µg/dL)
Ferritin	Severity will not be assessed Normal range (11 - 204 ng/mL)

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

± Modified to correspond to normal institutional laboratory ranges

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

B. Hematology

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin (Female) - gm/dL±	11.0 – 11.9	9.5 – 10.9	<9.5
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	>2.0
Hemoglobin (Male) - gm/dL±	12.5 – 13.6	10.5 – 12.4	<10.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	>2.0
WBC Increase - cell/mm$^{3\pm}$	9,900 – 15,000	15,001 – 20,000	>20,000
WBC Decrease - cell/mm^3	2,500 – 3,100	1,500 – 2,499	<1,500
Lymphocytes Decrease – cells/mm^3	500 – 599	400 – 499	<400
Neutrophils Decrease – cell/mm^3	1,500 – 1,999	1,000 – 1,499	<999
Platelets Decreased - cell/mm$^{3\pm}$	125,000 – 149,000	100,000 – 124,000	<100,000
PT – increase by factor (prothrombin time)	>1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	>1.20 x ULN
PTT – increase by factor (partial thromboplastin time)	>1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	>1.40 x ULN
Fibrinogen increase - mg/dL±	436 – 500	501 – 600	>600
Fibrinogen decrease - mg/dL±	150 – 212	125 – 149	< 125

* 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 the SDMCC to demonstrate that they are appropriate.

± Modified to correspond to institutional laboratory ranges

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

APPENDIX B

Case definitions of probable and confirmed myocarditis, pericarditis, and myopericarditis

Condition	Definition	
Acute myocarditis	**Probable case**	**Confirmed case**
	Presence of ≥ 1 new or worsening of the following clinical symptoms:*	Presence of ≥ 1 new or worsening of the following clinical symptoms:*
	chest pain, pressure, or discomfort	chest pain, pressure, or discomfort
	dyspnea, shortness of breath, or pain with breathing	dyspnea, shortness of breath, or pain with breathing
	palpitations	palpitations
	syncope	syncope
	OR, infants and children aged < 12 years might instead have ≥ 2 of the following symptoms:	OR, infants and children aged < 12 years might instead have ≥ 2 of the following symptoms:
	irritability	irritability
	vomiting	vomiting
	poor feeding	poor feeding
	tachypnea	tachypnea
	lethargy	lethargy
	AND ≥ 1 new finding of	AND ≥ 1 new finding of
	troponin level above upper limit of normal (any type of troponin)	Histopathologic confirmation of myocarditis†
	abnormal electrocardiogram (ECG or EKG) or rhythm monitoring findings consistent with myocarditis§	cMRI findings consistent with myocarditis¶ in the presence of troponin level above upper limit of normal (any type of troponin)
	abnormal cardiac function or wall motion abnormalities on echocardiogram	
	cMRI findings consistent with myocarditis¶	
	AND	AND
	No other identifiable cause of the symptoms and findings	No other identifiable cause of the symptoms and findings
Acute pericarditis**	Presence of ≥ 2 new or worsening of the following clinical features:	
	acute chest pain††	
	pericardial rub on exam	

	new ST-elevation or PR-depression on EKG
	new or worsening pericardial effusion on echocardiogram or MRI
Myopericarditis	This term may be used for patients who meet criteria for both myocarditis and pericarditis.

Abbreviations: AV = atrioventricular; cMRI = cardiac magnetic resonance imaging; ECG or EKG = electrocardiogram.

* Persons who lack the listed symptoms but who meet other criteria may be classified as subclinical myocarditis (probable or confirmed).

† Using the Dallas criteria (Aretz HT, Billingham ME, Edwards WD, et al. Myocarditis. A histopathologic definition and classification. Am J Cardiovasc Pathol 1987; 1:3–14). Autopsy cases may be classified as confirmed clinical myocarditis on the basis of meeting histopathologic criteria if no other identifiable cause.

§ To meet the ECG or rhythm monitoring criterion, a probable case must include at least one of 1) ST-segment or T-wave abnormalities; 2) Paroxysmal or sustained atrial, supraventricular, or ventricular arrhythmias; or 3) AV nodal conduction delays or intraventricular conduction defects.

¶ Using either the original or the revised Lake Louise criteria.
https://www.sciencedirect.com/science/article/pii/S0735109718388430?via%3Dihub

** https://academic.oup.com/eurheartj/article/36/42/2921/2293375

†† Typically described as pain made worse by lying down, deep inspiration, or cough, and relieved by sitting up or leaning forward, although other types of chest pain might occur.