A Phase I Trial to Evaluate the Safety and Immunogenicity of an Influenza Vaccination Strategy Including a H3N2 M2SR Prime followed by a Seasonal Quadrivalent Inactivated Vaccine Boost in a Pediatric Population 9-17 years old

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 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 312 (Investigational New Drug Application, 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: Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)), including the latest finalized revision
- International Conference on Harmonisation: Good Clinical Practice (ICH E6): 62 Federal Register 25691 (1997); and future revisions
- 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
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

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

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Daniel F. Hoft, MD, PhD
Principal Investigator

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LIST OF ABBREVIATIONS

AdvantageEDC^{SM}	Electronic Data Capture System
AE	Adverse Event/Adverse Experience
AESI	Adverse Event of Special Interest
BP	Blood Pressure
BPM	Beats Per Minute
CAR	Clinical Agents Repository
CBC	Complete Blood Count
CFC	Chlorofluorocarbons
CFR	Code of Federal Regulations
CFSE	Carboxyfluorescein Succinimidyl Ester
CI	Confidence Interval
CMI	Cell-Mediated Immunity
CROMS	Clinical Research Operations & Management Support
CSR	Clinical Study Report
°C	Degrees Celsius
°F	Degrees Fahrenheit
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DRM	Data Review Meetings
eCRF	Electronic Case Report Form
EIA	Enzyme-Linked Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunosorbent Spot
ELLA	Enzyme-Linked Lectin Assay
EPT	End Point Titer
FDA	Food and Drug Administration
FWA	Federalwide Assurance
g/dL	Grams per Deciliter
GBS	Guillain-Barré Syndrome
GMFR	Geometric Mean Fold Rise
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
HGB	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus

IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
IFN-γ	Interferon Gamma
IFU	Instructions for Use
IgG	Immunoglobulin G
IIV	Inactivated Influenza Virus Vaccine
IM	Intramuscular
IN	Intranasal
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-Treat
KG	Kilogram
LAIV	Live Attenuated Influenza Vaccine
mcg	Microgram(s)
µL	Microliter(s)
MDCK	Madin Darby Canine Kidney cells
MedDRA®	Medical Dictionary for Regulatory Activities
mg/dL	Milligram(s) per Deciliter
mL	Milliliter(s)
Mm	Millimeter(s)
mmHg	Millimeters of Mercury
MOP	Manual of Procedures
N	Number of Subjects
NA	Neuraminidase
NACL	Sodium Chloride
Neut	Neutralizing or Neutralization
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NOCMC	New Onset Chronic Medical Conditions
NIH	National Institutes of Health
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OER	Office of Extramural Research
OHRP	Office for Human Research Protections
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PHI	Personal Health Information

PP	Per Protocol
QA	Quality Assurance
QC	Quality Control
QIV	Quadrivalent Influenza Vaccine
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical Data Coordinating Center
sIgA	Secretory IgA
SLUCVD	Saint Louis University Center for Vaccine Development
SMC	Safety Monitoring Committee
SOB	Shortness of Breath
SOP	Standard Operating Procedure
[redacted]	[redacted]
TBD	To Be Determined
US	United States
USDA	US Department of Agriculture
VTEU	Vaccine and Treatment Evaluation Unit
WB	Whole Blood
WBC	White Blood Cells
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase I Trial to Evaluate the Safety and Immunogenicity of an Influenza Vaccination Strategy Including an H3N2 M2SR Prime followed by a Seasonal Quadrivalent Inactivated Vaccine Boost in a Pediatric Population 9-17 years old
Phase:	I
Population:	50 males and non-pregnant females, 9 to 17 years old, inclusive, who are in good health and meet all eligibility criteria
PNumber of Sites:	1 Vaccine and Treatment Evaluation Unit (VTEU) site
Study Duration:	Approximately 28 months
Subject Participation Duration:	Approximately 13 months
Description of Agent:	One dose of a monovalent live attenuated influenza H3N2 M2SR vaccine manufactured by FluGen or placebo administered intranasally (IN) followed by one dose of licensed quadrivalent influenza vaccine (QIV) administered intramuscularly 3 months later.
Objectives:	**Primary:** Safety: • To assess the safety and reactogenicity of a monovalent live attenuated influenza H3N2 M2SR vaccine. **Secondary:** Immunogenicity: • Identification of circulating and mucosal antibody responses induced by H3N2 M2SR vaccination.

- Identification of cellular immune responses induced by H3N2 M2SR vaccination.

Exploratory:

- Investigation of H3N2 M2SR priming effects on QIV-induced broadly neutralizing serum antibody responses to HA and NA.
- Investigation of H3N2 M2SR priming effects on QIV-induced influenza-specific secretory IgA (sIgA) responses.
- Investigation of H3N2 M2SR priming effects on QIV-induced influenza-specific CD4 and CD8 T cell lymphoproliferative and effector cytokine responses.

Outcome Measures:

Primary:

Safety:

- Frequency of solicited upper respiratory and systemic reactogenicity through approximately Day 8 after the first study vaccination.
- Frequency of unsolicited non-serious adverse events from the time of study vaccination through 21 days after the first study vaccination.
- Frequency of SAEs, regardless of seriousness or relationship to study vaccination, from receipt of the first vaccination throughout the entire study.
- Frequency of New Onset Chronic Medical Conditions (NOCMC) and AESIs that develop from receipt of vaccination through 3 months after first study vaccination.

Secondary:

Immunogenicity:

- For HAI and Neutralizing antibody responses, percentage of subjects achieving seroconversion (defined as either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40 or a pre-vaccination titer ≥1:10 and a minimum four-fold rise in post-vaccination antibody titer) against an H3N2 M2SR-like virus and H3N2 QIV-like viruses at approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- For HAI and Neutralizing antibody responses, percentage of subjects achieving a serum antibody titer of 1:40 or greater against the H3N2 M2SR-like virus and the H3N2 QIV-like virus at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- For HAI and Neutralizing antibody responses, the geometric mean titers (GMTs) and geometric mean fold rise (GMFR) to H3N2 M2SR-like virus and the H3N2 QIV-like virus at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- GMT and GMFR of nasal sIgA responses directed against the H3N2 M2SR virus and the H3N2 QIV virus measured by EPT(ELISA) with and without normalization to total sIgA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- Frequency of influenza H3 HA-specific (H3 M2SR-like and QIV-like viruses) and conserved internal viral protein-

specific IFN-γ ELISPOT responses at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

Exploratory:

- GMT and GMFR of serum chimeric HA ELISA reactivity with Groups 1 and 2 HA stem domains of the viral hemagglutinin at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased seroconversion rates and/or 4fold rises in either M2SR or QIV matched H3HA-specific HAI and/or MN assays induced by H3N2 M2SR vaccination).

- GMT and GMFR of serum anti-NA antibody titers to N1 and N2 Neuraminidases as determined by NA ELLA or ELISA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased seroconversion rates and/or 4-fold rises in either M2SR or QIV matched H3HA-specific HAI and/or MN assays induced by H3N2 M2SR vaccination).

- GMT and GMFR of nasal wash sIgA chimeric HA ELISA to the HA stem domains of the viral hemagglutinin (Group 2 versus Group 1 viruses with and without normalization to total sIgA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased GMT and/or GMFR of sIgA reactive with M2SR matched H3HA induced by H3N2 M2SR vaccination).

- GMT and GMFR of sIgA anti-NA antibody titers to N1 and N2 Neuraminidases as determined by NA ELLA or ELISA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased GMT and/or GMFR of sIgA reactive with M2SR matched H3HA induced by H3N2 M2SR vaccination.

- CD4+ and CD8+ T cell responses to the H3HA present in the M2SR vaccine and pools of highly conserved Influenza A T cell epitopes by 7 day CFSE dilution/flow cytometry at baseline and approximately 7, 21, and 56 days, following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased frequencies of H3HA-specific and/or conserved peptide epitope-specific IFN-gamma producing T cells induced by M2SR vaccination in ELISPOT assays).

Description of Study Design: This is a Phase I randomized, double-blind, comparison trial in 50 children and adolescents, 9 to 17 years old, inclusive, who are in good health and meet all eligibility criteria. This clinical trial is designed to assess the safety and immunogenicity of a monovalent H3N2 M2SR vaccine (manufactured by FluGen) in an older pediatric population. The monovalent influenza H3N2 M2SR virus vaccine was derived from influenza strain A/Brisbane/10/2007-like H3N2 (H3N2 M2SR).

Subjects will be enrolled in one of two groups in a 1:1 ratio as indicated in Table 1. Subjects will be randomly assigned to either receive one dose of Saline intranasally or one dose of M2SR intranasally followed by one dose of licensed QIV 3 months later.

Reactogenicity will be measured for the first vaccine (first vaccination) by the occurrence of solicited reactions from the time of study vaccination through Day 8 after vaccination. Unsolicited non-serious AEs will be collected from the time of the first study

vaccination through approximately 21 days after the first study vaccination. SAEs will be collected from the time of the first study vaccination throughout the entire study. AESIs (wheezing, and otitis media) and NOCMC will be documented and reported from the time of first study vaccination through approximately 3 months after first study vaccination.

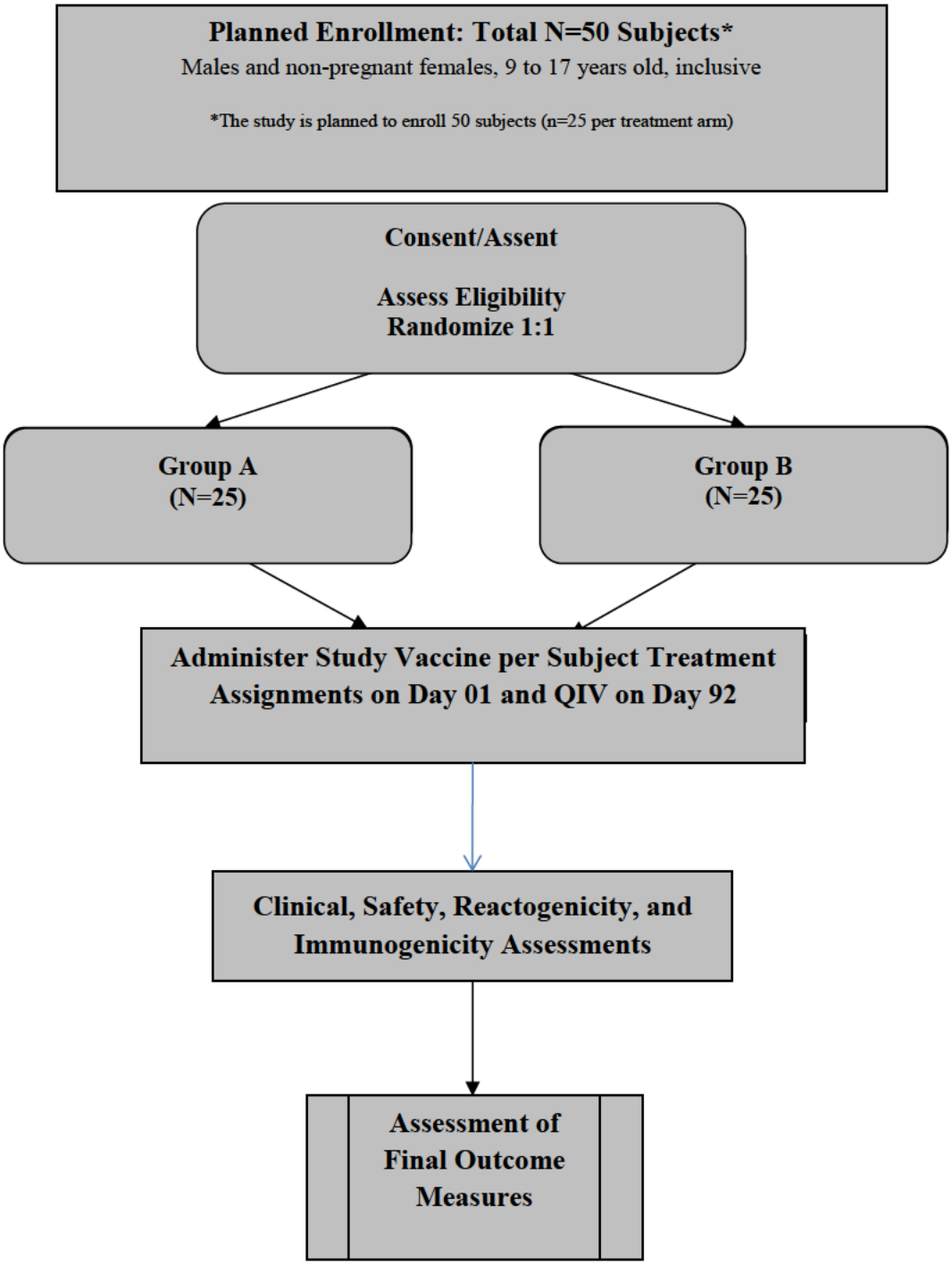
Immunogenicity testing will include performing HAI, HA/NA ELISA, neutralization, nasal secretory IgA (sIgA), IFN-γ ELISPOT and CFSE dilution/ICS assays. Serum for HAI, HA/NA ELISA and neutralization assays will be obtained on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd study vaccination (Day 92), and on Day 22 after the 2nd vaccination. Nasal swabs for sIgA will be collected on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd vaccination (Day 92), and on Day 22 after the 2nd vaccination. PBMC for T cell functional assays will be harvested on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to 2nd vaccination (Day 92), and on Day 22 after the 2nd vaccination.

Estimated Time to Complete Enrollment: Approximately 3 months

Table 1: Treatment Arms:

Treatment Arm	Subjects	First Study Vaccination	Second Study Vaccination
Group A	**25**	**M2SR**	**QIV**
Group B	**25**	**Placebo**	**QIV**

Figure 1: Schematic of Study Design:



1. KEY ROLES

Lead Principal Investigator:	Daniel F. Hoft, MD, PhD Saint Louis University Medical School
DMID Clinical Project Manager:	Tena Knudsen, RN Division of Microbiology and Infectious Diseases NIAID, NIH
Statistical Data Coordinating Center:	The Emmes Company, LLC

2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

Influenza continues to be a major threat to human health. Every year, thousands of persons in the US die from complications of influenza infection. Usually the elderly, young children and pregnant women are particularly susceptible to severe complications, but with some pandemic strains, over-exuberant inflammation in response to lower respiratory infection seems to disproportionately affect the middle aged and younger adult populations. Effective vaccines have been available for the past 50 years, but must be given annually to optimally protect against the diversity of new strains that circulate over time.

The influenza genome contains 8 segments, and each is prone to high error rates during viral replication. The high mutation rate results in extensive genetic diversity, especially within the surface proteins Hemagglutinin (HA) and Neuraminidase (NA). These molecules are constantly under intense immune pressure as major targets for viral specific, immunoprotective antibodies. The random mutation or genetic drift, combined with the selection of immune escape variants, renders vaccine immunity -involving primarily HA/NA specific antibodies- of only transient efficacy against the seasonally emerging strains that disseminate worldwide. Therefore, current vaccines targeting primarily the induction of HA-reactive antibodies specific for seasonal circulating strains must be administered yearly to be maximally effective. In years when the vaccines poorly matches the predominantly circulating strains, significant increased influenza morbidity and mortality can occur. Additionally, some groups (elderly and young children) respond less well than healthy adults to HA antigen when it is injected as vaccine.

Two major types of influenza vaccines have been developed and widely utilized. The inactivated influenza vaccines (IIV) contain primarily HA protein (and some variable NA protein levels that co-purify with HA during manufacture). The HA is derived from 3 (trivalent vaccine) or 4 (quadrivalent vaccine [QIV]) strains representing the major early circulating viral pathogens each season. IIV induce HA type-specific antibodies, measurable in highly standardized hemagglutination inhibition (HAI) in vitro assays. An HAI titer of at least 1:40 is known to be associated with protection against influenza strains expressing the specifically targeted matching HA sequences. IIV can be highly protective if given annually to young and middle-aged populations but are less effective in infants, the elderly and the immunosuppressed. Higher dose IIV, IIV including adjuvants and novel vaccines have been designed to enhance immunity in these special populations.

Live attenuated influenza vaccines (LAIV) are reassortment viruses generated with genomic segments expressing nonstructural proteins with attenuating mutations and HA/NA-encoding segments from seasonally circulating viral strains. Flumist, the only previously licensed LAIV, is delivered intranasally. Work led by Dr. Belshe and our SLU Center for Vaccine Development (SLUCVD) demonstrated that LAIV vaccination was more effective in children than IIV vaccination[1]. The improved efficacy in children is most likely due to the induction of a better combination of serum antibodies, secretory IgA (sIgA) and cell-mediated immunity (CMI); i.e., a more complete immune response than is seen in children given IIV. In adults LAIV is also effective, however several comparative studies have suggested equal efficacy or a modest advantage to IIV. Of interest, HA-specific serum HAI responses induced by LAIV in adults have not been highly predictive of protective immunity, further suggesting that either sIgA and/or CMI can be important. In addition, inducing antibodies directed against highly conserved HA stem epitopes instead of highly variable HA head epitopes is another possible explanation for the improved protection that has been induced by LAIV. Prime/boost strategies using 2 different heterotypic pandemic influenza vaccines have led to more broadly reactive antibody responses, and is another promising area for influenza vaccine research focusing on the development of universally protective novel vaccines.

The additional immune responses induced by LAIV responsible for increased protection in children, and protection in adults in the absence of increased serum HAI antibodies, are unclear. As mentioned above, LAIV has been shown to induce T cell responses and sIgA which likely are involved. Dr. Hoft's lab has recently shown that LAIV induces influenza-specific CD4+, CD8+ and γδ T cell responses in children not induced by IIV in children, including T cells specific for highly conserved epitopes encoded by internal/nonstructural viral proteins[2]. In addition, because LAIV is given intranasally, it also induces mucosal immune responses not induced by IIV. In fact, Dr. Hoft's lab has recently shown in adults that compared with IIV, LAIV uniquely induces influenza-specific sIgA responses, without inducing increased serum HAI responses or increased T cell responses[3]. Therefore, depending on the population being vaccinated, serum HAI antibodies, broadly protective HA stem-specific antibodies, T cell responses and/or sIgA all can be important for LAIV-induced protective immunity. In contrast, single or multiple IIV vaccinations appear to induce predominantly serum HAI antibodies specific for highly variable HA head-specific epitopes in the absence of broadly protective HA stem-specific antibody, T cell or sIgA responses.

2.2. Rationale

FluGen has produced new whole influenza virus M2SR vaccines which can be safely delivered intranasally. Because of M2 protein deletion, these M2SR vaccines undergo only a single

replication cycle after vaccination. The pre-clinical data from mice and ferrets are very promising, suggesting that M2SR vaccines can induce both humoral and cellular immune responses important for broadly protective influenza-specific immunity[4,5]. In addition, there is some evidence that M2SR vaccines may work better than Flumist vaccines for inducing broadly protective immune responses against antigenically-drifted viruses and heterotypic viruses expressing different HA subtypes. The fact that the immunogenicity of the M2SR vaccines does not appear to depend on sustained in vivo replication suggests that these vaccines could be more effective than Flumist in partially immune individuals.

The first in human phase 1 dose escalation trial of a monovalent H3N2 M2SR vaccine conducted in healthy adult subjects has demonstrated that the vaccine is generally safe and well-tolerated and that it is immunogenic. This protocol is a phase 1 study of the monovalent H3N2 M2SR vaccine in an older pediatric population, one of the most important vaccine targets for limiting influenza transmission from a public health point of view. In addition, we propose to study whether H3N2 M2SR priming elicits broadly protective HA stem-specific antibodies, NA antibodies, T cell responses and sIgA responses that are readily boosted by a seasonal QIV vaccine. Our hypotheses are that pediatric subjects primed with the M2SR vaccine will have more robust serum HAI and NA antibody responses, broadly neutralizing serum antibody responses, sIgA responses and cell-mediated immunity directed against both matched and unmatched influenza viruses. These immune responses could greatly advance public health protection against pandemic influenza, bringing us much closer to universal influenza vaccine-induced protection.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks

The potential risks of this trial are those associated with having blood drawn, possible reactions to the monovalent influenza H3N2 M2SR virus vaccine or the licensed inactivated QIV, and breach of confidentiality.

H3N2 M2SR Vaccine

Intranasal LAIV vaccines have induced nasal edema, nasal dryness, nasal pain/irritation, nasal congestion, rhinorrhea, decreased appetite, irritability, decreased activity, fatigue, malaise, sore throat (pharyngitis), difficulty swallowing, headache, arthralgia, myalgia, fever, feverishness (chills, shivering, sweating), cough, abdominal pain, nausea, vomiting, diarrhea, ear infections, conjunctivitis, itchy eyes, flushing, sinusitis, sneezing, nasal bleeding, shortness of breath, allergy, and wheezing/rhonchi in young children. Rarely, nosebleeds can occur after intranasal

vaccinations. Because H3N2 M2SR vaccine is a live attenuated vaccine given intranasally similar to other LAIV, we will specifically study these symptoms as solicited AEs.

Placebo

The placebo is physiological saline or 0.9% sodium chloride for injection. Side effects do not typically occur from administration of this solution to the nasal passages. If the subject's nasal passages are extremely dry or irritated it is possible they may experience a stinging sensation or have some mild rhinitis.

Licensed QIV

Occasionally, recipients of unadjuvanted licensed, QIVs may develop influenza-like reactions, such as fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), arthralgia (joint pain), headache, and/or nausea. Some subjects may develop reactions at the injection site, including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and/or tenderness. Most of these reactions peak in intensity in the first 24 hours after vaccination and disappear without treatment within 1 or 2 days. Analgesics (e.g., acetaminophen, or ibuprofen or similar non-steroidal anti-inflammatory drugs (NSAIDs) and rest may generally relieve or lessen these reactions. Bruising can sometimes occur due to the vaccination procedure.

In addition, post-marketing surveillance indicates AESIs (autoimmune, auto-inflammatory and immune-mediated diseases) as potential risks identified for the seasonal influenza vaccines including, but not limited to, neuritis, convulsions, severe allergic reactions, syncope, encephalitis, thrombocytopenia, vasculitis, and Guillain-Barré syndrome. Reports of these reactions were rare; however, exact incidence rates cannot be precisely calculated.

Allergic Reaction

Acute and potentially life-threatening allergic reactions are also possible. Very rarely, occurring in about 1 in 4 million people given a vaccination, there can be a serious allergic reaction to a vaccine. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or loss of blood pressure (hypotension). If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur.

Blood Draw

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the blood draw site for a few minutes after the blood is taken.

Intramuscular Injection

Intramuscular injection may also cause transient discomfort and fainting. Giving an IM injection may also cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn or where the study vaccination will be given extremely unlikely.

Nasal Swabs

Obtaining a nasal swab may cause mild discomfort and on rare occasions may cause a nosebleed.

Pregnancy

It is unknown if this vaccine poses any risks to an unborn child. As such, female adolescents of childbearing potential, i.e., women who have started menses, must agree to use an effective method of birth control for at least 30 days prior to first study vaccination and for at least 30 days following the second vaccination. Males who are sexually active with a female of childbearing potential must agree not to father a child for 30 days after receipt of the first study vaccination.

Other Risks

In addition, post-marketing surveillance of seasonal influenza vaccines identified other adverse events including, but not limited to, neuritis, convulsions, severe allergic reactions, syncope, encephalitis, thrombocytopenia, vasculitis, and Guillain-Barré syndrome. Reports of these reactions were rare; however, exact incidence rates cannot be precisely calculated.

During the swine influenza (H1N1) vaccine campaign of 1976, some recipients developed a paralytic illness called Guillain-Barré syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza (H1N1) vaccine at about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines. Most persons who develop GBS recover completely, although the recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack. Intensive surveillance of GBS after administration of inactivated influenza vaccines since 1976 has shown a slight increase in risk over background cases (more

than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination[6]. Interestingly, although vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine-associated GBS have declined[7]. A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older[8]. An active, population-based surveillance study conducted in the United States during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines[9-11]. Another study using the Medicare system showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48)[12]. An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS[13]. It is unknown if the administration of the H3N2 M2SR and seasonal vaccines to be used in this study will result in the incidence of GBS as high as that seen with the 1976 vaccine product as the mechanism leading to this response has not been completely elucidated.

PHI

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU site. Electronic files will be password-protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Any publications from this trial will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU site for quality assurance and data analysis include groups such as the local Institutional Review Board (IRB) NIAID and FDA.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify subjects. At most, this web site will include a summary of the results.

There may be other risks, discomforts, or side effects that are unknown at this time.

2.3.2. Known Potential Benefits

There are no known benefits attributable to the receipt of the monovalent live attenuated H3N2 M2SR virus vaccine. It is anticipated that vaccination using the monovalent live attenuated

H3N2 M2SR may result in some protection against infection caused by the influenza H3N2 virus. The duration and type of protection elicited in humans is currently unknown. The monovalent live attenuated H3N2 M2SR virus vaccine may offer protection against circulating seasonal influenza viruses, but this has not yet been demonstrated in humans. It is likely that the subject will develop at least partial protective immunity from receiving the licensed QIV depending on the match between vaccine virus and circulating viruses. It is also possible that if the subject gets sick from flu infection, symptoms or complications would be less severe than if they had not been vaccinated. Society might benefit in the future from the information gathered during this study as it may help researchers better understand how to improve influenza vaccines.

3. OBJECTIVES AND OUTCOME MEASURES

3.1. Study Objectives

3.1.1. Primary:

Safety:

- To assess the safety and reactogenicity of a monovalent live attenuated influenza H3N2 M2SR vaccine.

3.1.2. Secondary:

Immunogenicity:

- Identification of circulating and mucosal antibody responses induced by H3N2 M2SR vaccination.
- Identification of cellular immune responses induced by H3N2 M2SR vaccination.

3.1.3. Exploratory:

- Investigation of H3N2 M2SR priming effects on QIV-induced broadly neutralizing antibody responses to HA and NA.
- Investigation of H3N2 M2SR priming effects on QIV-induced influenza-specific secretory IgA (sIgA) responses.
- Investigation of H3N2 M2SR priming effects on QIV-induced influenza-specific CD4 and CD8 T cell lymphoproliferative and effector cytokine responses.

3.2. Study Outcome Measures

3.2.1. Primary:

Safety:

- Frequency of solicited upper respiratory and systemic reactogenicity through approximately Day 8 after the first study vaccination.

- Frequency of unsolicited non-serious adverse events from the time of study vaccination through 21 days after the first study vaccination.

- Frequency of SAEs, regardless of seriousness or relationship to study vaccination, from receipt of the first vaccination throughout the entire study.

- Frequency of New Onset Chronic Medical Conditions (NOCMC) and AESIs that develop from receipt of vaccination through 3 months after first study vaccination.

3.2.2. Secondary:

Immunogenicity:

- For HAI and Neutralizing antibody responses, percentage of subjects achieving seroconversion (defined as either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40, or a pre-vaccination titer ≥1:10 and a minimum four-fold rise in post-vaccination antibody titer) against H3N2 M2SR-like and H3N2 QIV-like viruses at approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- For HAI and Neutralizing antibody responses, percentage of subjects achieving a serum antibody titer of 1:40 or greater against the H3N2 M2SR-like virus and the H3N2 QIV-like virus at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- For HAI and Neutralizing antibody responses, the geometric mean titers (GMTs) and geometric mean fold rise (GMFR) to H3N2 M2SR-like virus and H3N2 QIV-like virus at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- GMT and GMFR of nasal sIgA responses directed against the H3N2 M2SR virus and the H3N2 QIV virus measured by EPT(ELISA) with and without normalization to total sIgA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

- Frequency of influenza H3 HA-specific (H3 M2SR-like and QIV-like viruses) and conserved internal viral protein-specific IFN-γ ELISPOT responses at baseline and

approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV.

3.2.3. Exploratory:

- GMT and GMFR of serum chimeric HA ELISA reactivity with Groups 1 and 2 HA stem domains of the viral hemagglutinin at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased seroconversion rates and/or 4-fold rise as measured by GMFR in either M2SR or QIV matched H3HA-specific HAI and/or MN assays induced by H3N2 M2SR vaccination.)

- GMT and GMFR of serum anti-NA antibody titers to N1 and N2 Neuraminidases as determined by NA ELLA or ELISA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased seroconversion rates and/or 4-fold rises in either M2SR or QIV matched H3N2-specific HAI and/or MN assays induced by H3N2 M2SR vaccination).

- GMT and GMFR of nasal wash sIgA chimeric HA ELISA to the HA stem domains of the viral hemagglutinin (Group 2 versus Group 1 viruses with and without normalization to total sIgA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased GMT and/or GMFR of sIgA reactive with M2SR matched H3HA induced by H3N2 M2SR vaccination).

- GMT and GMFR of sIgA anti-NA antibody titers to N1 and N2 Neuraminidases as determined by NA ELLA or ELISA at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of QIV. (Note: These studies will be contingent upon seeing increased GMT and/or GMFR of sIgA reactive with M2SR matched H3HA induced by H3N2 M2SR vaccination).

- CD4+ and CD8+ T cell responses to the H3HA present in the M2SR vaccine and pools of highly conserved Influenza A T cell epitopes by 7 day CFSE dilution/flow cytometry at baseline and approximately 7, 21, and 56 days following receipt of the first study vaccination and immediately prior to and approximately 21 days following receipt of

QIV. (Note: These studies will be contingent upon seeing increased frequencies of H3HA-specific and/or conserved peptide epitope-specific IFN-gamma producing T cells induced by M2SR vaccination in ELISPOT assays).

4. STUDY DESIGN

This is a Phase I double-blind, randomized, placebo-controlled study in 50 healthy adolescents and children, 9-17 years of age, who are in good health and meet all eligibility criteria. The study is designed to assess the safety and immunogenicity of a prime-boost regimen of H3N2 M2SR intranasal influenza vaccine followed by licensed inactivated QIV boost administered IM. Group A will receive one dose of M2SR intranasally on Day 1 and one dose of QIV on Day 92. Group B will receive one dose of saline intranasally on Day 1, and one dose of QIV on Day 92. See Table 1 for the randomization scheme for this trial.

The two treatment arms will be enrolled and vaccinated concurrently.

Reactogenicity will be measured by the occurrence of solicited upper respiratory, respiratory tract assessment, and systemic reactions from the time of the experimental (first) study vaccination through 7 days after vaccination. Unsolicited non-serious AEs will be collected from the time of the first study vaccination through approximately 21 days after the first study vaccination. SAEs will be collected from the time of the first study vaccination throughout the entire study. AESIs (wheezing, and otitis media) and NOCMC will be documented and reported from the time of first study vaccination through approximately 3months after first study vaccination.

Immunogenicity testing will include performing HAI, HA/NA ELISA, neutralization, nasal secretory IgA (sIgA), IFN-γ ELISPOT and CFSE dilution/ICS assays. Serum for HAI, HA/NA ELISA and neutralization assays will be obtained on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd study vaccination (Day 92), and on Day 22 after the 2nd vaccination. Nasal swabs for sIgA will be collected on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd vaccination (Day 92, and on Day 22 after the 2nd vaccination. PBMC for T cell functional assays (IFN-γ ELISPOT and CFSE dilution/ICS assays) will be harvested on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to 2nd vaccination (Day 92), and on Day 22 after the 2nd vaccination.

The duration of this study for each subject will be approximately 13 months.

5. STUDY ENROLLMENT AND WITHDRAWAL

Approximately 50 males and non-pregnant females, 9 to 17 years old, inclusive, who are in good health and meet all eligibility criteria, will be enrolled from 1 VTEU site participating in this trial. The target population should reflect the community at large at the participating VTEU site. Estimated time to complete enrollment in this trial is approximately 3 months for the 2018/2019 season and 3 months for the 2019/2020 season. Information regarding this trial may be provided to potential subjects who have previously participated in vaccine trials conducted at the participating VTEU site. Other forms and/or mechanisms of recruitment may also be used. The local Institutional Review Board (IRB) will approve all materials prior to use.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

No exemptions are granted on Subject Inclusion or Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer.

5.1. Subject Inclusion Criteria

Subjects eligible to participate in this trial must meet all of the following inclusion criteria:

1. Parent(s)/legal guardian(s) must provide written informed consent prior to initiation of any study procedures, and subject must provide assent.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Are males or non-pregnant females, 9-17 years old, inclusive at the time of enrollment.
4. Are in good health[1].

[1]*As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, ER, or urgent care for condition and no adverse symptoms that need medical intervention). This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition 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. Any change in prescription medication due to* ***improvement*** *of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, 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 principal*

investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Low dose topical steroids, herbals, vitamins, and supplements are permitted.

5. Oral temperature is less than 100.0°F.

6. For female adolescent of child-bearing potential[2] must agree to correctly use an acceptable method of contraception[3] from 30 days prior to vaccination until 30 days after the last study vaccination.

[2]Defined by the onset of menses, and not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating l).

[3]Includes non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, and correct use of male condoms with the use of applied spermicide, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, contraceptive patches or oral contraceptives ("the pill"). Method of contraception will be captured on the appropriate data collection form.

7. Female adolescent of childbearing potential must have a negative urine pregnancy test within 24 hours prior to study vaccination.
8. Males who are sexually active with a female of childbearing potential must agree not to father a child for 30 days after receipt of the first study vaccination.
9. Agrees not to participate in another clinical trial during the study period.
10. Agrees not to donate blood or blood products to a blood bank for 12 months after receiving the investigational vaccine.
11. Weight $\geq$ 34 kg or 75 pounds.
12. Hemoglobin $\geq$ 11.5 g/dL.
13. Hematocrit > 35%.
14. Ferritin level $\geq$ 15 ng/mL.
15. Parent/legal guardian must provide consent to future use of stored samples.

5.2. Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Have an acute illness[4], as determined by the site PI or appropriate sub-investigator, within 72 hours prior to each study vaccination.

   [4] *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[5].

   [5] *Including acute or chronic medical disease or condition, defined as persisting for at least 90 days, 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 study.*

3. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.

4. Have known active neoplastic disease or a history of any hematologic malignancy. Non- melanoma skin cancers that are not active are permitted.
5. Have known HIV, hepatitis B, or hepatitis C infection.
6. Have a history of severe reactions following previous immunization with licensed or unlicensed influenza vaccines.
7. History of anatomic disorder of the nares or nasopharynx (Deviated septum is allowed).
8. History of chronic sinus infections.
9. Have a history of Guillain-Barré Syndrome.
10. Have a history of alcohol or drug abuse prior to study vaccination.
11. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.
12. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others prior to study vaccination.
13. Have taken oral and/or nasal corticosteroids of any dose within 30 days prior to each study vaccination or plan to take in the 30 days following the first study vaccination.

14. Have taken high-dose[7],[8] inhaled corticosteroids within 30 days (prior to study vaccination).

[7] *High-dose defined as per age as using inhaled high dose per reference chart*

[8] *https://www.nhlbi.nih.gov/files/docs/guidelines/asthma_qrg.pdf.*

15. Use of aspirin or salicylate-containing products 30 days prior to the first vaccination.

16. Recurring or active wheezing or diagnosis of asthma, or history of significant wheezing[9]

[9] *Medically significant wheezing: defined as wheezing on physical exam plus sign of respiratory distress (tachypnea, retractions, or dyspnea), hypoxemia (02 saturation < 95%), or new bronchodilator prescription, or use of daily bronchodilator therapy (not on an "as needed" basis).*

17. Received any licensed live or inactivated vaccine within 30 days prior to or plan to receive any licensed live or inactivated vaccine within 30 days after each study vaccination.

18. Received any influenza vaccine (inactivated or live) within 6 months prior to the first study vaccination and until the end of the study.

19. Received immunoglobulin or other blood products within 6 months prior to study vaccination.

20. Received an experimental agent[10] within 30 days prior to the first study vaccination, or expects to receive an experimental agent[11] during the 12-month trial-reporting period.

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

[11] *Other than from participation in this study.*

21. Are participating or plan to participate in another clinical trial with an interventional agent[12] that will be received during the 12-month trial-reporting period.

[12] *Including agent (licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication) during the 12- month study period.*

22. Prior history of H3N2 actual or potential exposure or infection prior to the first study vaccination, or receipt of experimental vaccines within the past year prior to the first study vaccine.

23. Female adolescent subject who is breastfeeding or plans to breastfeed at any given time from the first study vaccination until 30 days after the last study vaccination.

24. Blood donation within 30 days prior to the study vaccination through 30 days after the last blood drawn for this study.

25. Have signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity[13].

[13] *The study vaccination should be postponed/deferred until signs or symptoms have resolved and if within the acceptable protocol-specified window for that visit.*

26. Have received any antiviral drug within 3 days of study vaccination.

5.3. Treatment Assignment Procedures

5.3.1. Randomization Procedures

Conformity with the ICHE6; and per FDA Guidance for Industry E6: Good Clinical Practice (GCP), Consolidated Summary; screening records will be kept at the participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical Data Coordinating Center's (SDCC) AdvantageEDC^{SM} (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. Fifty subjects will be randomly assigned to one of two treatment arms, as shown in Table 1. The list of randomized treatment assignments will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. AdvantageEDC^{SM} will assign each subject to a treatment arm after the demographic and eligibility data have been entered into the system. A designated individual at the VTEU site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the AdvantageEDC^{SM} User's Guide. Manual back-up procedures and instructions are provided for use in the event that the VTEU site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subjects who are randomized but do not receive study vaccine may be replaced. Subjects who are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up will not be replaced.

See Table 1 for the randomization scheme for this trial.

5.3.2. Masking Procedures

This is a double-blind clinical trial.

Subjects, investigators, study personnel performing any study-related assessments following study vaccine administration will be blinded to treatment assignment, laboratory personnel performing immunologic and future-use research assays will be blinded to treatment assignment, subject ID, and visit number.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., research pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the participating VTEU site.

The unblinded study vaccine administrator is a study personnel licensed, registered, or certified to administer medications/vaccines, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

The Safety Monitoring Committee (SMC) may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment arm be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only.

5.3.3. Reasons for Withdrawals and Discontinuation of Study Product Administration

Parent(s)/legal guardian(s) may voluntarily withdraw their consent for trial participation at any time and for any reason, without penalty.

A subject may withdraw or be withdrawn from this trial for any of the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of this trial, or would interfere with the evaluation of responses.
- Subject no longer meets eligibility criteria (see Section 5.1).
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Withdrawal of consent.
- Lost to follow-up.

- Termination of this trial.
- New information becomes available that makes further participation unsafe.

The second study vaccination will <u>not</u> be administered to a subject if any of the following criteria are met:

- Meets the contraindication on the package insert to receipt of licensed influenza vaccine.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject/parent(s)/legal guardian(s) refusal of further study vaccination.
- Withdrawal of consent.
- Subject is lost to follow-up.
- Termination of this trial.
- New information becomes available that makes further participation unsafe.

5.3.4. Handling of Withdrawals and Discontinuation of Administration

The primary reason for withdrawal from this trial will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 8.

Although subjects are free to withdraw at any time or may be withdrawn by the site principal investigator or appropriate sub-investigator at any time (see Section 5.3.3), those subjects who receive only one dose of study vaccine will be encouraged to remain in this trial for follow-up safety assessments (may be conducted by phone call/electronic communication (e.g., email, text message) rather than in person) continuing through approximately 3 months after their first study vaccination. These subjects will also be encouraged to provide a venous blood sample for immunogenicity and future-use research assays on all follow-up study visits. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Every attempt will be made to follow all AEs, including solicited dose site and systemic reactions, unsolicited non-serious AEs, SAEs ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of AE.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls, emails, text messages, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's study records.

5.3.5. Subject Replacement

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced. Over enrollment (randomized per the study design) may be allowed if any subjects withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine. Over enrollment must be approved by the sponsor.

5.3.6. Termination of Study

Although the sponsor has every intention of completing this trial, it reserves the right to terminate this trial at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to SMC review and recommendation and at the discretion of DMID.

6. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

[redacted]

Investigational Vaccine (Bris10 M2SR)

The Bris10 M2SR vaccine is provided as a [redacted] of M2SR encoding the HA and NA of influenza virus strain A/Brisbane/10/2007 (H3N2). The virus is [redacted]

Vaccine Diluent ([redacted])

M2SR vaccine will be [redacted]. Each vial of M2SR vaccine and diluent is considered to be a single-use vial. The diluted vaccine will be aseptically transferred by the pharmacist to the ≈ nasal spray devices, [redacted] for administration to the subject. Additional detail is supplied in the MOP.

Licensed Inactivated QIV

FLUCELVAX® QIV is a licensed inactivated influenza vaccine for intramuscular use and is formulated to contain a total of 60 mcg hemagglutinin (HA) per 0.5 mL dose. It is prepared from virus propagated in Madin Darby Canine Kidney (MDCK) cells, a continuous cell line.

These cells were adapted to grow freely in suspension in culture medium. The virus is inactivated with β-propiolactone, disrupted by the detergent, cetyltrimethylammonium bromide and purified through several process steps. Each of the 4 virus strains is produced and purified separately then pooled to formulate the quadrivalent vaccine.

Placebo (Normal Saline)

The 0.9% sodium chloride for injection, USP, is a sterile, nonpyrogenic, isotonic solution of sodium chloride. It is preservative-free and supplied in a single-dose container. The placebo will be aseptically transferred by the pharmacist into the nasal spray Vaxinator or into the MAD301 nasal sprayer device (provided by FluGen) for administration to the subject. Additional detail is supplied in the MOP.

VaxINator

The VaxINator™ is made from radiation-stable medical-grade polycarbonate material and is compliant with USP Class VI and ISO 10993 requirements. Investigational vaccine or placebo will be administered nasally via the VaxINator atomization device attached to a 1 mL syringe. The VaxINator is manufactured by Teleflex Medical. The VaxINator is a conical shape plastic component that Luer-locks onto a standard syringe. The VaxINator atomizes the liquid vaccine as it exits the syringe. The conical shape forms a plug in the nostril and high applied-pressure atomizes the liquid into a fine mist.

MAD301 Sprayer Device

The MAD301 atomization device is made from radiation-stable medical-grade polycarbonate material and is compliant with USP Class VI and ISO 10993 requirements. Investigational vaccine or placebo will be administered nasally via the MAD301 atomization device attached to a 1 mL syringe. The MAD301 sprayer is manufactured by Teleflex Medical. The MAD301 is a conical shape plastic component that Luer-locks onto a standard syringe. The MAD301 atomizes the liquid vaccine as it exits the syringe. The conical shape forms a plug in the nostril and high applied-pressure atomizes the liquid into a fine mist.

6.1.1. Acquisition

Bris10 M2SR vaccine is manufactured and provided by FluGen.

VaxINator kits and the MAD301 sprayer devices will be shipped from FluGen Inc (Madison, WI) to the Fisher BioServices repository for distribution to the clinical site.

Upon DMID authorization, the M2SR vaccine, [redacted] diluent, and the VaxINator and the MAD301 sprayer devices will be transferred to the following address:

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

Normal saline and FLUCELVAX® will be obtained by the DMID Clinical Materials Services (CMS), Fisher BioServices.

All study products (i.e., M2SR, [redacted] buffer, normal saline, the VaxINator and the MAD301 sprayer devices) will be shipped to the participating VTEU site prior to the start of this study upon request and with prior approval from DMID. FLUCELVAX® will be shipped to the participating VTEU site once available. Should the site principal investigator require additional study vaccine or study products during this trial, further instructions are provided in the protocol-specific MOP.

6.1.2. Formulation, Packaging, and Labeling

Investigational Vaccine (Bris10 M2SR)

The investigational vaccine, Bris10 M2SR, is provided at a [redacted] Each vial is a single-use vial. The vaccine is an infectious but non-replicating influenza virus. [redacted] Refer to the Investigator's Brochure and supplements for the M2SR as well as in the protocol specific MOP.

Vaccine Diluent [redacted]

The vaccine diluent, [redacted] is used to prepare dilutions of the investigational

vaccine for the active dose treatments. Each vial is a single-use vial. The unblinded pharmacist will remove a vial from [redacted] will be used to prepare the diluted vaccine stock solutions for the H3N2 M2SR treatment group.

Vials (used and unused) will be [redacted] and retained until after accountability is completed by the Sponsor for disposal.

Licensed Inactivated QIV

FLUCELVAX® Quadrivalent Influenza Vaccine from Seqirus Inc. is a sterile, slightly opalescent suspension in phosphate buffered saline and will be provided in 0.5 mL single-dose prefilled Luer Lock syringes that contain no preservatives or antibiotics. It is stored at refrigerator temperature 2° to 8°C (35° to 46°F). Do not freeze.

Used syringes will not be retained after administration.

Refer to the FLUCELVAX® package insert as well as the protocol specific MOP.

Placebo (Normal Saline)

The placebo consists of physiological saline (containing no preservatives and suitable for intranasal administration) and is a clear, colorless solution. It will be sourced by DMID CMS in single-use containers. It is stored at ambient temperature 20°C to 25°C (68°F to 77°F) (See USP Controlled Room Temperature). The unblinded pharmacist will aseptically withdraw volumes of the placebo as needed to fill the nasal spray devices.

The placebo container(s) will be retained until after accountability is completed by the Sponsor for disposal.

VaxINator

VaxINator kits are supplied in individual sterile pouches containing a VaxINator atomization device, a 1-mL single-use syringe, and a dose divider (not utilized in the current protocol and will be discarded). VaxINator devices are stored at 20°C to 25°C (68°F to 77°F) (See USP Controlled Room Temperature).

MAD301 sprayer devices

MAD301 atomization devices are supplied in individual non-sterile pouches and are attached to sterile 1-mL single-use syringes before use (see below). MAD301 sprayer devices are stored at 20°C to 25°C (68°F to 77°F) (See USP Controlled Room Temperature).

Refer also to the VaxINator Kit and the MAD301 sprayer device Instructions For Use (IFU) as well as in the protocol-specific MOP.

Each of these study products except the VaxINator and the MAD301 nasal sprayer, will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use."

6.1.3 Product Storage and Stability

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays as applicable), continuously monitored and recorded during the duration of this trial per the participating VTEU 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 principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected study product(s) must not be administered. The site principal investigator 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 manufacturer 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 are provided in the protocol-specific MOP.

6.2. Dosage, Preparation, and Administration of Study Intervention/ Investigational Product

See the protocol-specific MOP Appendices for detailed information on the preparation, labeling, storage and administration of study vaccine for each group. Study vaccine preparation will be performed by the participating VTEU site's pharmacist on the same day of study vaccine administration.

Investigational Vaccine (Bris10 M2SR)

Visually inspect the study vaccine (Bris10 M2SR, vaccine diluent and placebo [normal saline]) upon receipt and prior to preparation and use. If the study vaccine appear(s) to have been damaged, contaminated or discolored, or if there are any concerns regarding its integrity, do NOT use the affected study vaccine. The affected study vaccine must be quarantined and labeled as "Do Not Use (until further notice)." The Site Principal Investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study vaccine can be used. If it cannot be used, the site will receive specific final disposition instructions from DMID. If the study vaccine is unusable, the participating VTEU site's pharmacist will prepare another dose. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Depending on randomization, each subject will be randomized to receive a one-time intranasal administration of a priming vaccine either:

Bris10 M2SR vaccine at 10^8 virus particles OR placebo (normal saline) at Day 1 followed by a one-time intramuscular administration of quadrivalent inactivated vaccine at Day 92.
The Bris10 M2SR vaccine will be diluted on-site as needed by the Pharmacist. The Pharmacist will also fill vaccine and placebo into nasal spray devices.

Study vaccine administration will be performed by an unblinded study vaccine administrator who is credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. On the assigned days, each dose of study vaccine or placebo will be administered to subjects via the nasal spray devices into each nostril.

Filling and masking the nasal spray device

M2SR vaccine and placebo will be prepared and filled into a Teleflex VaxINator nasal spray device (Figure 2) by the unblinded pharmacist. For both Groups A and B, two devices will be filled per subject to deliver 150 µl volume to each nare; a total of 300 µl.

Figure 2: Image of the VaxINator Device

M2SR vaccine and placebo will be prepared and filled into a sterile 1 mL polypropylene syringe that is fitted with a Teleflex MAD301 sprayer for intranasal delivery (Figure 3) by the unblinded pharmacist. For both Groups A and B, two devices will be filled per subject to deliver 150 µl volume to each nare; a total of 300 µl.

Figure 3: Image of the MAD301 Sprayer Device

To fill the device, the contents are drawn into the syringe barrel via a draw-up tube. For the Teleflex VaxINator the draw-up tube is removed and replaced with the nose cone via a Luer lock fitting. For the MAD301, the draw-up tube is removed and replaced with the MAD301 sprayer via a Luer lock fitting. The device is then primed. Further details for filling and actuating the device are provided in the MOP. The pharmacist will wrap an opaque label completely around the circumference of the syringe barrel to conceal any color of the liquid contents and will use an indelible marker to record the subject's ID and device number (#1 or #2) on the label. The pharmacist or other clinic staff will then use the masked, filled device #1 to administer the first half of the dose (active or placebo) to the subject, followed by use of the masked, filled device #2 to administer the second half of the dose to the same subject. The device number will be provided by the research pharmacist.

Short-term Stability

Stability of the M2SR product has been tested and informs the following conditions:

- [redacted]

- [redacted]

[redacted]

[redacted]

6.3. Modification of Study Intervention/Investigational Product for a Subject

There will be no dose modifications. If a subject's second study vaccination is deferred, it should be rescheduled to occur within the acceptable protocol-specified window for that visit (Day 92±14 days post first study vaccination). If a protocol deviation occurs due to unforeseen circumstances related to the second vaccination window and the QIV cannot be given within the protocol window, the DMID Medical Officer must approve the second vaccination and the documentation of approval should be filed in the subject's chart.

Subjects who do not receive the second study vaccination will be encouraged to provide a venous blood sample for immunogenicity and future research testing. See Sections 5.3.3 and 5.3.4 for reasons for and handling of withdrawals and discontinuation of treatment. See the protocol-specific MOP for alternate follow-up requirements.

6.4. Accountability Procedures for the Study Intervention/ Investigational Product(s)

After receipt of the Bris10 M2SR vaccine, [redacted] diluent, QIV, and normal saline placebo, the site principal investigator is responsible for study product distribution and disposition, and has ultimate responsibility for study product accountability. The site principal investigator may delegate to the participating VTEU site's research pharmacist responsibility for study product accountability. The participating VTEU site's research pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study product(s). The study product accountability records and dispensing logs will also capture vial numbers, date of study vaccine preparation/administration, time of study product removal from storage freezer, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study

product(s), including the amount of Bris10 M2SR, [redacted] diluent, QIV and normal saline, 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 VTEU site's study product accountability records and dispensing logs per the site monitoring plan.

Used and unused vials of Bris10 M2SR and [redacted] diluent, and normal saline will be retained until monitored and released for disposition as applicable. After dosing subjects, remnants of Bris10 M2SR vaccine doses [redacted] until returned to FluGen for dose confirmation. Unused syringes of QIV will be stored at recommended temperature for neuraminidase quantitation. Dose confirmation and quantitation can occur on an ongoing basis for used vials of Bris10 M2SR and QIV. Final disposition of the unused Bris10 M2SR, QIV, and normal saline will be determined by DMID and communicated to the participating VTEU site by the DMID Clinical Project Manager.

6.5. Assessment of Subject Compliance with Study Intervention/ Investigational Product/Investigational Device

Study product will be administered to subjects by an unblinded study vaccine administrator via intranasal route for Dose 1 and IM injection for Dose 2 according to subject treatment assignment and as described in Section 6.4. Thus, subject compliance is not anticipated to be an issue.

6.6. Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken in the 30 days prior to signing the informed consent form through approximately 21 days after the first study vaccination, early termination or unscheduled visits (if prior to 21 days after the first study vaccination), whichever occurs first. Medications reported in the electronic case report form (eCRF) are limited to those taken within 30 days prior to the first study vaccination through approximately 21 days after the first study vaccination. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. In addition, receipt of non-study influenza vaccines will be solicited through approximately 21 days after the first study vaccination, and reported in the eCRF. The use of aspirin or salicylate containing products will be solicited from 30 days prior to the first vaccination through 21 days after the last vaccination and reported in the eCRF.

7. STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4 and 7 and Appendix A: Schedule of Study Procedures and Evaluations.

7.1. Recruitment

The targeted/planned enrollment of subjects by gender and ethnicity/race will be distributed appropriately based on the Inclusion/Exclusion criteria of the protocol and will also be representative of the population of the region. The VTEU is committed to accessing the diverse population of the region for enrollment into the study while taking into account the protocol specific criteria.

Subjects will be recruited actively and prospectively. Methods of recruitment may include but are not limited to letters, postcards, email messages, handouts, brochures, websites, posters/flyers, public service announcements, press releases, interviews with the media, Facebook posts, Twitter feeds, contact by telephone, lectures, announcements, and discussions held in groups or individually.

IRB approved recruitment materials will be posted to the SLU Media Relations and Center for Vaccine Development Facebook Page and SLU Media Relations Twitter Feed. For those parents/legal guardians who follow our Facebook page and Twitter feed, they will see the posted recruitment information. Our email address will be provided so they can contact us for more information if interested.

7.2. Screening Visit

7.2.1. Visit 00, Screening Visit

(Window: Day -28 to -1)

- Parent(s)/legal guardian(s) will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures. Subject will be provided with an age appropriate assent information sheet that includes a description of the trial (purpose and study procedures). The information will be reviewed with the subject and the subject must provide assent prior to performing any study procedures.

- Demographic information will be obtained by interview of parent(s)/legal guardian(s).

- Eligibility criteria will be reviewed with parent(s)/legal guardian(s).

- Complete medical history will be obtained by interview of parent(s)/legal guardian(s) to ensure eligibility.

- All concomitant medications taken within 30 days prior to signing the informed consent form will be reported on the eCRF.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- Subject receipt of licensed seasonal influenza vaccine over the previous two seasons, what type (inactivated or live attenuated), and approximate date of vaccination will be recorded on the appropriate data collection form, if known. Prior receipt of licensed seasonal influenza vaccine is not exclusionary, as long as it has been administered within the allowable window (see Section 5.2).

- Subject receipt of non-seasonal influenza vaccines, including those that are experimental, what type (inactivated or live attenuated), what subtype (e.g., A/H3, A/H5, A/H9) and approximate date of vaccination will be recorded on the appropriate DCF, if known. Prior receipt of non-seasonal influenza vaccines are not exclusionary, except influenza A/H7 vaccines (see Section 5.2).

- Weight will be obtained.

- Oral temperature will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A physical examination will be performed on all subjects to include the following organs and organ systems: skin, head and neck including upper respiratory tract (nares and nasopharynx), lungs including wheezing, heart, liver, spleen, extremities, lymph nodes, and nervous system, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Age and developmentally appropriate counseling about pregnancy prevention may be provided.

- A urine pregnancy test will be performed on all female adolescent subjects of childbearing potential and must be negative to ensure eligibility.

- Approximately 14 mL of venous blood will be collected for clinical evaluation and safety labs including WBC, HGB, HCT, Platelet Count, ALT, AST, Serum Creatinine, Ferritin, PT

and aPTT.

7.3. Enrollment Visit

7.3.1. Visit 01, Enrollment and First Study Vaccination, (Dose 1), Clinic Visit

- Eligibility criteria will be reviewed with parent(s)/legal guardian(s).
- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form prior to the second study vaccination.
- Subject receipt of licensed seasonal influenza vaccine over the previous two seasons, what type (inactivated or live attenuated), and approximate date of vaccination will be recorded on the appropriate data collection form, if known. Prior receipt of licensed seasonal influenza vaccine is not exclusionary, as long as it has been administered within the allowable window (see Section 5.2).
- Subject receipt of non-seasonal influenza vaccines, including those that are experimental, what type (inactivated or live attenuated), what subtype (e.g., A/H3, A/H5, A/H9) and approximate date of vaccination will be recorded on the appropriate DCF, if known. Prior receipt of non-seasonal influenza vaccines are not exclusionary, except influenza A/H7 vaccines (see Section 5.2).
- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.
- Oral temperature will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A limited physical examination to specifically include the nares (including edema), throat, and lungs (including evaluation for wheezing) will be performed and a targeted physical examination may be performed on the other body systems if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A nasal swab for sIgA will be obtained.
- Age and developmentally appropriate counseling about pregnancy prevention may be provided.
- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.
- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays.
- A urine pregnancy test will be performed on all female adolescent subjects of childbearing potential and must be negative to ensure eligibility.
- Subjects will be enrolled in AdvantageEDC^{SM} and assigned randomly to a treatment arm prior to the first study vaccination.
- Pre-administration reactogenicity assessments will be performed prior to the first study vaccination to establish baseline. Subjects will then receive a single dose of study product or placebo intranasally. Subjects will be observed in the clinic for at least 30 minutes after the first study vaccination. Post-administration reactogenicity assessments will be completed, and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.
- Parent(s)/legal guardian(s) will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited systemic reactions, any unsolicited AEs, and concomitant medications. Parent(s)/legal guardian(s) will be encouraged to take child's oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Parent(s)/legal guardian(s) will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Parent(s)/legal guardian(s) will be instructed to notify the study center if they develop any severe reactions after the first study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the parent(s)/legal/guardian(s) on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.4. Follow-up

Follow-up visits are scheduled in reference to study vaccination dates as indicated for each visit window.

7.4.1. Visit 02, Day 08, Clinic Visit (Window: 7±1 days post first study vaccination)

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.
- Subject's parent(s)/legal guardian(s) will be asked if the subject had any additional blood taken for medical reasons.
- Memory aid information will be reviewed with parent(s)/legal guardian(s).
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form prior to the second study vaccination.
- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.
- All AE/AESIs/NOCMCs/SAEs will be recorded on the appropriate data collection form.
- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.
- A limited physical examination to specifically include the nares (including edema), throat, and lungs (including evaluation for wheezing) will be performed and a targeted physical examination may be performed on the other body systems if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- A nasal swab for sIgA will be obtained.
- Approximately 14 mL of venous blood will be collected for safety labs including WBC, HGB, Platelet Count, ALT, AST, Serum Creatinine, PT and aPTT.
- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.

- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays.

7.4.2. Visit 03, Day 22, Clinic Visit (Window: 21±2 days post first study vaccination)

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.

- Subject or subject's parent(s)/legal guardian(s) will be asked if the subject had any additional blood taken for medical reasons.

- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form prior to the second study vaccination.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination. All AE/AESIs/NOCMCs/SAEs will be recorded on the appropriate data collection form.

- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A nasal swab for sIgA will be obtained.

- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.

- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays.

- Approximately 4 mL of venous blood will be collected if Hgb drops $\geq$ 0.5g/dL or < lower limits of normal from screen to Day 8, draw repeat Hgb.

7.4.3. Visit 04, Day 57, Clinic Visit (Window: 56±3 days post first study vaccination)

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.

- Subject or subject's parent(s)/legal guardian(s) will be asked if the subject had any additional blood taken for medical reasons.

- AEs limited to AESIs, NOCMCs, and SAEs will be recorded on the appropriate data collection form.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- A nasal swab for sIgA will be obtained.

- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.

- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays.

- Approximately 4 mL of venous blood will be collected if Hgb level at Day 22 has dropped 0.5 g/dL or more from baseline or is < lower limit of normal. If results at Day 57 still meet that criteria ($\geq$0.5 g/dL drop from baseline or < lower limit of normal), no further PBMC collection will be done. If results have recovered to within 0.4 g/dL of baseline at Day 57, resume blood collection plan for the rest of the study.

7.4.4. Visit 05, Day 92, Second Vaccination (Dose 2), Clinic Visit (Window: 91±14 days post first study vaccination)

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.
- Criteria for administering QIV will be reviewed with parent(s)/legal guardian(s).
- Concomitant medications (limited to aspirin or salicylate containing products) will be recorded on the appropriate data collection form.
- All AESIs/NOCMCs/SAEs will be recorded on the appropriate data collection form. Reporting of AEs occurring since previous visit but before 2nd vaccination is limited to AESIs, NOCMCs, and SAEs.
- A targeted physical examination may be performed prior to the second study vaccination, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.
- Oral temperature will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A urine pregnancy test will be performed on all female adolescent subjects of childbearing potential and must be negative to ensure eligibility.
- A nasal swab for sIgA will be obtained.
- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.
- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays. Note: PBMCs will not be collected if the HgB was consistently low through Day 57.
- Subjects will receive their second vaccination IM in whichever deltoid they prefer.

- Subjects will receive a single dose of study vaccine via IM injection into the deltoid muscle of the preferred arm. The site of injection and time of administration will be recorded on the appropriated data collection form. Subjects will be observed in the clinic for at least 30 minutes after the second study vaccination.

7.4.5. Visit 06, Day 113, Clinic Visit (Window: 21±2 days post second study vaccination)

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.

- Concomitant medications (limited to aspirin or salicylate containing products) will be recorded on the appropriate data collection form.

- All SAEs will be recorded on the appropriate data collection form.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- A nasal swab for sIgA will be obtained.

- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.

- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays. Note: PBMCs will not be collected if the HgB was consistently low through Day 57.

- Approximately 8 mL of venous blood will be collected for Hgb and Ferritin level. If the ferritin level is < 15 ng/mL and/or the Hgb is less than the lower limits of normal, recommend follow-up with primary care provider for consideration of multivitamins with iron.

7.4.6. Visit 07; Day 240, Phone Call

(Window: 148±14 days post second vaccination)

- Study personnel will contact parent(s)/legal guardian(s) by phone to solicit any SAEs, medical history information that have occurred since the previous clinic visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

7.5. Final Study Visit, Visit 08, Day 366, Phone Call

(Window: 274±14 days post second vaccination)

- Study personnel will contact parent(s)/legal guardian(s) by phone to solicit any SAEs, medical history information that have occurred since the previous clinic visit will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

7.6. Early Termination Visit (if needed)

The following activities will be performed at the early termination visit on subjects who withdraw, or are withdrawn or terminated from this trial:

- Interim medical history, including an assessment for new medical conditions will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with parent(s)/legal guardian(s) (if within 8 days after the last study vaccination).

- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form (if within 21 days after the first study vaccination). If within 30 days of either vaccination through 21 days after the last vaccination, aspirin or salicylate containing products will be recorded.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- All AE/AESIs/NOCMCs/SAEs will be recorded on the appropriate data collection form. AEs will be limited to AESIs and SAEs if after 21 days the first study vaccination. AESIs and NOCMCs will be collected until 3 months and SAEs will be collected throughout the entire study.

- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- Oral temperature may be obtained if indicated. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A limited physical examination to specifically include the nares (including edema), throat, and lungs will be done if within 8 days after first vaccination.

- A targeted physical examination may be performed prior to the second study vaccination, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A nasal swab for sIgA will be obtained.

- Approximately 6 mL of venous blood will be collected for serum HAI, HA/NA ELISA and neutralization assays.

- Approximately 16 mL of PBMCs will be collected for IFN-γ ELISPOT and CFSE dilution/ICS assays. Note: PBMCs will not be collected if the HgB was consistently low through Day 57.

7.7. Unscheduled Visit (if needed)

Unscheduled visits may occur at any time during this trial. Any of the following activities may be performed:

- Interim medical history will be obtained by interview of parent(s)/legal guardian(s) and any changes since the previous clinic visit or contact will be noted (if indicated).

- Memory aid information will be reviewed with parent(s)/legal guardian(s) (if within 8 days after the first study vaccination).

- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form (if within 21 days after the first study vaccination). If within 30 days of either vaccination through 21 days after the last vaccination, aspirin or salicylate containing products will be recorded.

- Subject's/parent(s)/legal guardian(s) will be counseled on the avoidance of taking any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- All AE/AESIs/NOCMCs/SAEs will be recorded on the appropriate data collection form. AEs will be limited to AESIs, NOCMCs, and SAEs if after 21 days after the first study vaccination. AESIs and NOCMCs will be collected until 3 months and SAEs will be collected throughout the entire study.

- In the event that the subject attends the visit without a parent/legal guardian, the parent/legal guardian will be called to collect updated medical history, concomitant medications, and will be reminded that the subject is to avoid any aspirin or salicylate containing products for 30 days prior to receipt of the first vaccination through 21 days after the last vaccination.

- Oral temperature may be obtained if indicated. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A limited physical examination to specifically include the nares (including edema), throat, and lungs will be performed if within 7 days after vaccination.

- A targeted physical examination may be performed prior to the second study vaccination, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

8. STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Complete medical history will be obtained by interview of subjects, parent(s) and/or legal guardian(s) on the Screening visit. Subjects, parent(s) and/or legal guardian(s) will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited at follow-up visits after the first study vaccination, an interim medical history will be obtained by interview of subjects, parent(s) and/or legal guardian(s) noting any changes since the previous clinic visit or contact.

See section 6.6 for the collection of medication history. Use of new medication should prompt evaluation for the occurrence of any AESIs or NOCMC. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Sections 5.1and 5.2). In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

- At the screening visit a physical examination will be performed on all subjects to include the following organs and organ systems: including skin, head and neck including upper respiratory tract (nares and nasopharynx), lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. At Visit 01, 02 and Early Termination or Unscheduled Visits if they occur within 8 days of vaccination after the first study vaccination, a limited physical examination to include the nares (including edema), throat, and lungs will be performed and a targeted physical examination may be performed on the other body systems, if indicated based on the subject's interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. At all other follow-up visits, a targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

Oral temperature will be collected prior to each study vaccination. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Reactogenicity assessments will include an assessment of solicited AEs occurring from the time of experimental (first) study vaccination through Day 8 after vaccination. For upper respiratory symptoms, this includes an assessment of runny nose, stuffy nose/ congestion, sneezing, nasal pain/irritation/nasal dryness, nasal bleeding/epistaxis, sinus pressure/pain, sore throat/sore/scratchy, itchy or painful throat, cough, trouble breathing/shortness of breath. For general systemic symptoms, this includes an assessment of reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise, myalgia (body aches/muscular pain), arthralgia (joint pain), headache, flushing, decreased activity, decreased appetite, abdominal pain, nausea, vomiting, diarrhea, eye pruritus, eye redness (conjunctivitis), and allergy. For respiratory tract symptoms, this will include an assessment of nares' erythema and induration.

Memory Aids: All parent(s)/legal guardian(s) will complete a subject memory aid from the time of the first study vaccination Day 01 (Visit 01) through 7 days after the first study vaccination. Memory aids will be reviewed with the parent(s)/legal guardian(s) for AEs at the clinic visit following receipt of first study vaccination. If a subject noted ongoing local (nasal or injection site) or systemic reactogenicity on the 7^{th} day following the study vaccination (study Day 8), the memory aid will continue to be reviewed until resolved. The memory aids will not be retained as a source document.

Subjects will be observed in the clinic for at least 30 minutes after each study vaccination. Any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.

8.2. Laboratory Evaluations

8.2.1. Clinical Laboratory Evaluations

Urine pregnancy tests will be performed locally by site laboratory at the Screening visit and within 24 hours prior to each study vaccination (Day 01 (Visit 01) and Day 92 (Visit 05) on all female adolescents of childbearing potential. Results must be negative and known prior to randomization on Day 1 and administration of each study vaccination to be eligible for participation in this study and receipt of each study vaccination.

- Clinical laboratory parameters to be evaluated at the Screening Visit (Visit 00) and on Day 08 (Visit 02) will include a White Blood Cell Count (WC), HGB, HCT (only at Visit 00), Platelet Count, ALT, AST, Serum Creatinine, Ferritin (at Visit 00), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT). The results from the clinical safety laboratory parameters collected on Visit 00 will be available and reviewed prior to study

vaccination. Results of the HGB and HCT must be within normal limits, and Ferritin must be ≥ 15 ng/mL for enrollment. HGB will also be repeated at Day 22 and/or Day 57 if HGB is below the lower limit of normal or ≥0.5g/dL below the baseline screening level at the day 8 follow up safety. HGB and Ferritin will be drawn at Day 113.

All other results can be and will serve as a Grade 1 or normal.

8.2.2. Special Assays or Procedures

Immunogenicity

Immunogenicity testing will include performing HAI, HA/NA ELISA, neutralization, nasal sIgA, IFN-γ ELISPOT and CFSE dilution/ICS assays and will be done by the Saint Louis University laboratory. Serum for HAI, HA/NA ELISA and neutralization assays will be obtained on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd study vaccination (Day 92), and on Day 22 after the 2nd vaccination. Nasal swabs for sIgA will be collected on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to the 2nd vaccination (Day 92), and on Day 22 after the 2nd vaccination. PBMC for T cell functional assays will be harvested on Day 01 (pre-vaccination), Days 08, 22, and 57 after the first vaccination, immediately prior to 2nd vaccination (Day 92), and on Day 22 after the 2nd vaccination. Subjects who withdraw early will have HAI and Neut antibody assays completed on available samples.

The volumes of venous blood to be collected for HAI, ELISA, neutralization assays, IFN-γ ELISPOT CFSE dilution/ICS assays, and future use assays are presented in the table below.

Table 2: Venipuncture Volumes (mL):

Study Visit Number	**VS00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**
Study Day post 1st study vaccination	**Screen**	**D01 Dose #1**	**D08**	**D22**	**D57**	**D92 Dose #2**	**D 113**	**D 240**	**D 366**
Study Day post 2nd study vaccination						**D01**	**D22**	**D 149**	**D 275**
Clinical Screening and Safety Laboratory Evaluations	14 mL[2,3,4,]		14mL[3]	4mL[5]	4mL[5]		8mL[4,6]		
Serum for HAI, HA/NA ELISA, Neutralization Assays		6 mL	6 mL	6 mL	6 mL	6 mL	6 mL		
Nasal Swab sIgA		X	X	X	X	X	X		
PBMC for IFN-γ ELISPOT CFSE Dilution/ICS Assays		16 mL	16 mL	16 mL	16 mL	16 mL	16 mL		
Total (mL)	14 mL	22mL[1]	36 mL[1]	26mL[1]	26mL[1]	22mL[1]	30mL[1]	0	0
Cumulative Total (mL)	14 mL	36 mL	72 mL	98 mL	124 mL	146 mL	176 mL	176 mL	176 mL

[1] Verify that for pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. (NIH blood draw policy) or institutional policy is followed.
[2] Includes HCT.
[3] Includes WBC, Hgb, Platelet Count, ALT, AST, Serum Creatinine, PT, and aPTT
[4]Includes Ferritin Level.
[5] Includes Hgb; Draw if Hgb drops ≥0.5 g/dL or < lower limits of normal at previous visit.
[6]Includes Hgb..

8.2.3. Specimen Preparation, Handling, and Shipping

8.2.3.1. Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

8.2.3.2. Specimen Shipment

Specimen shipment will occur at intervals during the course of this trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the protocol-specific MOP.

Specimens for HAI and Neut antibody assays will be shipped from the participating VTEU site to the DMID CMS.

Further instructions for specimen shipment are included in the protocol-specific MOP, as appropriate.

9. ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. SAEs occurring from the time of the first study vaccination through 12 months after the first vaccination.

2. AESIs (wheezing, otitis media) and NOCMCs occurring from the time of the first study vaccination through 3 months after the first vaccination.

3. Solicited Adverse Events – reactogenicity events occurring from the time of experimental (first) study vaccination through 7 days after the first study vaccination. See tables listed in section 9.2.2 and 9.2.6 for local upper respiratory, respiratory tract assessment, and systemic symptoms:

4. Unsolicited Non-Serious Adverse Events –non-serious AEs occurring from the time of the first study vaccination through approximately 21 days after the first study vaccination.

5. Clinical safety laboratory AEs occurring at approximately 7 days after the first vaccination. Parameters to be evaluated include a WBC, HGB, Platelet Count, ALT, AST, Serum Creatinine, Ferritin level, PT and aPTT. HGB will also be repeated at Day 22 if there is a drop in HGB level from screen to Day 8 of $\geq$ 0.5 g/dL or results are < lower limits of normal. If unable to collect at Day 22, collect at Day 57. If drawn at Day 22 and the results are still low, repeat at Day 57. If HGB results are still low at Day 57, no further PBMC collection will be done. HGB and Ferritin level will be drawn at Day 113. If the ferritin is < 15 ng/mL and/or the HGB is less than the lower limits of normal, will recommend follow-up with primary care provider for consideration of multivitamins with iron.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event (AE): ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the

use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited local (respiratory) and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator), date of resolution of the event, 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.

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 will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). 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.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

New-Onset Chronic Medical Conditions (NOCMCs): NOCMCs are defined as any new ICD-10 diagnosis that is applied to the subject during the duration 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.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system (see Sections 9.2.2 and 9.2.3). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.

- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.

- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product: The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2. Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (upper respiratory) and systemic (subjective and quantitative) reactions:

Local (Upper Respiratory) Reactogenicity Grading

Local (Nasal) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Runny Nose	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter

Local (Nasal) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Stuffy Nose/Congestion	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with breathing from nose	not being able to breath from nose, or prevents daily activity or seeks medical encounter
Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Nasal Pain/Irritation	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter
Nasal Bleeding/Epistaxis	Total duration of all episodes in a 24 hour period <30 minutes	Total duration of all episodes in a 24 hour period $\geq$ 30 minutes	Any bleeding that required visit for medical encounter
Sinus Pressure/Pain	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Sore Throat/Sore/Scratchy/ Itchy or Painful Throat	Noticeable but does not interfere with eating and/or drinking	Moderate discomfort. Interferes with eating and/or drinking	Significant discomfort/prevents eating and/or drinking or seeks medical encounter
Cough	Noticeable but does not interfere with daily activity or sleeping	Moderate discomfort/interferes with daily activity or sleeping	Significant discomfort/prevents daily activity or seeks medical encounter
Trouble breathing/Shortness of Breath	Noticeable but does not interfere with daily activity or not	Moderate discomfort/interferes with daily activity or	Significant discomfort/prevents daily activity or seeks

Local (Nasal) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
	troubled by breathlessness except on vigorous exercise	short of breath with regular movement activities such as when hurrying on the level or walking up a slight incline and need to stop	medical encounter or TOO breathless to leave the house, or breathless when undressing, preventing normal activities

Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (Chills/Shivering/Sweating)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue (Tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (General Unwell Feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (Joint Pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity and/or seeks medical encounter
Flushing	Asymptomatic flushing	Symptoms, some interference with daily activity	Symptomatic, significant interference, prevents daily activity
Decreased Activity	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Decreased Appetite	Loss of appetite without decrease in oral intake	Loss of appetite associated with decreased oral intake	Loss of appetite without oral intake, seek medical care
Abdominal Pain	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	Transient (<24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)
Vomiting	Transient or intermittent AND no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent vomiting, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Diarrhea	Transient or intermittent AND no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent diarrhea, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Eye Pruritus	Transient of intermittent or minimal interference and no intervention	Persistent or frequent episodes, some interference with daily activity	Significant symptoms, prevents daily activity, or seeks medical attention
Eye Redness (Conjunctivitis)	Asymptomatic eye redness	Symptomatic eye redness, some interference with daily activity	Eye redness prevents daily activity or seeks medical encounter
Allergy	Pruritus with or without rash, no medical intervention	Localized urticaria, with intervention	Generalized Urticaria, Anaphylaxis, or Angioedema

Oral temperature[#] will be graded as follows:

Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

[#] Oral temperature assessed on Day 1 prior to the first study vaccination will be considered as baseline.

* A fever can be considered not related to the study product if an alternative etiology can be documented.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

9.2.3. Serious Adverse Events

Serious Adverse Event (SAE):

An AE or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE*,
- 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 hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening AE. An AE is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution.
- Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

At any time after protocol follow up period or completion of this study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be

related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.2.4. Adverse Events of Special Interest (AESI)

- Medically significant wheezing: defined as wheezing on physical exam sign of respiratory distress (tachypnea, retractions, or dyspnea), hypoxemia (O_2 saturation < 95%), or new bronchodilator prescription, or use of daily bronchodilator therapy (not on an "as needed" basis) from receipt of the first vaccination through 3 months after the first vaccination. Will record presence or absence of any wheezing after V02 and whether wheezing is diffuse or localized, respiratory rate, oxygen saturation, medications associated with wheezing, and physician's description on auscultation.

- Otitis Media and Wheezing at V02 only will be graded as follows:

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Otitis Media	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Wheezing	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

9.2.5. Clinical Safety Laboratory Adverse Event Grading

Note: Ranges have been modified to reflect local laboratory normal ranges for the site conducting this study

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
WBC cells/mm3; 103 cells/µL (Decrease)	2.0 – 2.4	1.50 – 1.0	<1.5
HgB g/dL 9-12 years of age	9.5-10.4	8.5-9.4	<8.5

HgB g/dL Males ≥ 13 years of age	10.0 – 10.9	9.0 - < 10.0	< 9.0
HgB g/dL Females ≥ 13 years of age	9.5 – 10.4	8.5 - < 9.5	< 8.5
Platelets cell/mm^3/UL	100- < 125	50- < 100	< 50
PT	1.1 - <1.25 x ULN	1.25 - < 1.50 x ULN	≥ 1.50 x ULN
aPTT	1.1 - < 1.66 x ULN	1.66 - <2.33 x ULN	≥ 2.33 x ULN

Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
ALT	1.25 – < 2.5 x ULN	≥2.5 – <5.0 x ULN	≥5.0 x ULN
AST	1.25 – < 2.5 x ULN	≥2.5 – <5.0 x ULN	≥5.0 x ULN
Creatinine mg/dL	1.1 – 1.3 x ULN	>1.3 – 1.8 OR Increase to 1.3 to < 1.5 x subject's baseline	>1.8 x ULN OR Increase to ≥ 1.5 x subject's baseline

9.2.6. Respiratory Tract Assessment Grading: Erythema and Induration

Erythema and Induration of the nasal cavity will be graded as follows:

Parameter	**Grade 1**	**Grade 2**	**Grade 3**
Erythema (Redness)	Very slight or barely perceptible redness	Well defined redness	Moderate to severe beet redness to eschar

Parameter	Grade 1	Grade 2	Grade 3
Induration (Swelling or edema)	Very slight or barely perceptible swelling	Slight swelling (edges of area well-defined by definite raising)	Moderate swelling (edges raised approximately 1 mm)

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

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, regardless of the relationship to study product. AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

9.3. Reporting Procedures

Solicited upper respiratory assessment, respiratory tract assessment, and systemic reactogenicity events will be documented and reported from the time of experimental (first) study vaccination through 7 days after vaccination.

Unsolicited non-serious AEs will be documented and reported from the time of the first study vaccination through approximately 21 days after the first study vaccination.

SAEs will be documented and reported from the time of the first study vaccination through 12 months after the first vaccination.

AESIs (wheezing and otitis media) and NOCMCs will be documented and reported from the time of first study vaccination through approximately 3 months after first study vaccination.

9.3.1 Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

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 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: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into AdvantageEDC^{SM}. 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 site will send a copy of the SAE report(s) to the ISM (as deemed necessary) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID

Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. 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 this trial, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all participating site principal investigators (i.e., all principal investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of 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. 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 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.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3. Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC^{SM} on the Pregnancy Report form. No further study vaccinations will be administered to pregnant subjects, but with the subject's permission all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety for the duration of this trial. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

9.4. Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be collected, assessed, and followed through resolution from the time of each study vaccination through approximately 21 days after the first study vaccination.

AESIs and NOCMCs will be collected, assessed, and followed through resolution from the time of the first study vaccination through approximately 3 months after the first study vaccination. SAEs will be collected, assessed, and followed from the time of the first study vaccination through resolution even if this extends beyond the trial-reporting period (through 12 months after the first vaccination).

Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

If the site principal investigator or appropriate sub-investigator becomes aware of an acute febrile illness and the site principal investigator or appropriate sub-investigator decides to bring the subject in for an evaluation to determine etiology, then the site principal investigator or appropriate sub-investigator, at their own discretion, can determine if specific viral testing should be done by either culture or polymerase chain reaction (PCR) to determine if the infectious agent was influenza and what strain.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5. Halting Rules

Further enrollment and study vaccinations will be halted for SMC review/recommendation if any of the following are reported following experimental (first) vaccination:

- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Two or more subjects experience the same grade 3 Adverse Event of any kind after administration of study product through day 8 that is considered related to study product
- Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within 3 days after administration of study product that is considered related to study product.

- Any subject experiences a study vaccine-related SAE from the time of the first study vaccination through the subject's last study visit.

Grading scales for solicited upper respiratory and systemic (subjective and quantitative) reactions are included in Section 9.2.2.

If any of the halting rules are met following any subject receipt of the first 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.

DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire trial, as applicable.

9.6. Safety Oversight

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide a written report to DMID. Participation is for the duration of the DMID study. The ISM must meet the requirements of the NIAID conflict of interest polity.

The ISM:

- Is in close proximity to the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a Conflict of Interest (COI) certification at the time they are asked to participate and provide updates to this information as needed.

- Receive reports of Serious Adverse Events (SAEs) from the site investigator and will be notified by email when DMID is notified of the SAE.

- Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner using the report form and email the report to DMID-CROMS SOCS.

- Communicate with the investigator at the participating site as needed.

- Review additional safety related events at the request of DMID.

- Provide additional information to DMID and/or the DSMB by teleconference as requested.

9.6.2 Safety Monitoring Committee (SMC)

This study will utilize an SMC, which is an independent group of experts that advises DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or it there are concerns that arise during the study.

The SMC will review the safety data at the following milestones:

- Organizational meeting (prior to start of the study)

- Data Review Meetings (DRM) will be held during the study. The SMC will review and evaluate accumulated trial data for subject safety, trial conduct, and trial progress at the below time points. Unless a halting rule has been met or there is a safety concern, screening, enrollment, and vaccination will continue while the data is being prepared and reviewed by the SMC for these DRMs.

    - For Dose 1 – all available safety data through Day 8 after all subjects have received 1st vaccination.

- An ad hoc SMC meeting will be convened when a halting rule is met, or at the request of the investigator and/or DMID if there are safety concerns during the course of the study.

- Early analysis review: After clinical database lock on day 57 to review the cumulative unblinded safety data for this trial. The data will be provided in a standard summary format.

- Final Data Review Meeting: May be held 6 to 8 months after final clinical database lock to review the cumulative unblinded safety and immunogenicity data for this trial. If a final review meeting is held, the data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

All reviews by the SMC will be performed using unblinded data. Study drug administration data, including dose interruptions, modifications, and the associated reason(s), will be reported to the SMC.

The DMID Medical Monitor is empowered to stop study enrollment and vaccine administration if adverse events that meet the halting criteria are reported or if any serious safety concerns arise. The DMID Medical Monitor and the ISM will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during the study.

10. CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken, and will document site visit findings and discussions.

11. STATISTICAL CONSIDERATIONS

11.1 Study Hypothesis

This Phase I study is exploratory, rather than confirmatory and not designed to test a formal null hypothesis. Rather, it is intended to obtain preliminary data in a pediatric population to estimate the immune response induced by this vaccine and to uncover any safety issues that occur at a sufficiently high rate that they might be observed in a study of this size.

11.2. Sample Size Considerations

This study is planned to enroll approximately 25 subjects in each treatment arm (Table 1). The sample size for this study was selected to obtain preliminary estimates in a pediatric population. While this study is not designed to test any specific null hypothesis, the following tables illustrate the precision and power available for select estimates and comparisons of interest.

Table 3 indicates the probability of observing one or more safety events, such as solicited local or systemic reactogenicity events or an unsolicited AE of a particular type, for a single treatment arm (N=25), and for all enrolled subjects (N=50).

Table 3: Power (%) to Detect Safety Events:

Event Frequency	Single Treatment Arm N=25	All Enrolled Subjects N=50
10% Very Common	93	>99
1% Common	22	40
0.1% Uncommon	3	5
0.01% Rare	<1	<1

Table 4 illustrates the minimum detectable differences in the proportion of subjects responding (e.g., attaining seroconversion or a titer ≥1:40) between two treatment arms for a range of possible response rates in the comparator arm, using a Chi-Square Test and alpha = 0.05. In a Previous DMID trial (12-0016) in this population approximately 8% of subjects did not have available immunogenicity data at Day 42. Table 4 is presented for the planned enrollment (N = 25 per group) and accounting for 10% drop out (N = 22 per group).

Table 4: Minimum Detectable Difference in Proportion Responders with 80% Power

Assumed Proportion of Responders in Comparator Arm	N = 25	N = 22
0.20	0.36	0.40
0.40	0.36	0.40
0.60	0.31	0.33
0.80	0.20	0.20

11.3. Planned Interim Analyses

Immunogenicity and safety results will be provided for the purpose of planning subsequent trials as described in Sections 11.3.1 and 11.3.2. Interim analyses would only be used to terminate this trial in the event that unanticipated safety events deemed to be of sufficient concern require such action by the sponsor. These assessments will not be made on the basis of testing a formal statistical hypothesis; therefore, p-value adjustment will not be made to any analyses. An SMC will be convened by DMID to review study progress and participant, clinical, safety, and reactogenicity data.

11.3.1 Interim Safety Review

Reports for interim safety review may include enrollment, and demographic information, dosing, solicited and unsolicited AE/SAE data, and clinical laboratory results. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs, and may request the treatment assignment be unblinded for an individual subject if required for safety

assessment. The SMC will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

Additional safety data may be requested by the vaccine partner during study follow-up for the purpose of planning future trials. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Reports may include data summarized by unblinded treatment arm; if so, the presentation will be in a format that prevents inadvertent unblinding of any individual subject. Such reports will be made available to the sponsor and vaccine partner for planning subsequent trials. These analyses will not be used to make any decisions concerning the conduct of this trial. No hypothesis testing will be included in any interim report. Additionally, the study will be monitored to determine if any of the halting rules described in Section 9.5 are met.

11.3.2. Interim Immunogenicity Review

An interim report of blinded MN results will be provided after the 19 subjects enrolled in 2018 have completed the Day 113 visit. This streamlined report will be made available to the sponsor and vaccine partner for planning subsequent trials. These results will not be used to make any decisions concerning the conduct of this trial. No hypothesis testing will be included in any interim analysis.

11.4. Final Analysis Plan

Once the last subject completes the last immunogenicity outcome visit (approximately 21 days after the second study vaccination), all serum samples will be sent for secondary immunogenicity assays (HAI, MN, and sIgA). After such data are reported in the database, and the data are considered clean and complete, a “topline” subset of the immunogenicity tables planned for the CSR will be provided to DMID on an expedited timeline.

Once the last subject completes the last clinical visit (approximately 275 days after the second study vaccination), the clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, and immunogenicity will be performed by the SDCC after the primary clinical database is locked and all immunogenicity data are received.

Analysis of exploratory immunogenicity endpoints may be performed and released as the data are available from the research laboratory. Any such analyses would be considered the final analysis for the endpoint and included in the CSR. Additional exploratory immunogenicity

endpoint data not available at the time of CSR preparation may be included in an addendum to the CSR. A formal statistical analysis plan will be developed and finalized prior to clinical and laboratory database freeze for the interim analysis, which will define the analyses to be included in the interim analysis and the final CSR.

11.4.1. Analysis Populations

The Safety Analysis population includes all subjects who received at least one dose of study vaccine.

The modified intent-to-treat (mITT) population includes all subjects who received at least one dose of study vaccine and contributed both pre- and at least one post-study vaccination venous blood samples for immunogenicity testing (HAI antibody assays) for which valid results were reported. For analyses using the mITT population, subjects will be grouped based on randomized treatment arm.

The per protocol (PP) population includes all subjects in the mITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline.
- Data from all visits subsequent to major protocol deviations, such as:
- Second study vaccination not received,
- Second study vaccination received out of window,
- Receipt of non-study licensed live or inactivated vaccine within 30 days prior to the first study vaccination,
- Receipt of influenza vaccine (live or inactivated) within 6 months prior to the first study vaccination.
- Receipt of immunosuppressive therapy (e.g., corticosteroids)
    - Receipt of aspirin or salicylate-containing products
    - Receipt of antiviral therapy
- Data from any visit that occurs substantially out of window, such that in the opinion of the study investigator could affect the scientific goals of this protocol.

For analyses using the PP population, subjects will be grouped based on study vaccinations received.

11.4.2. Primary Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population.

Solicited AEs will be summarized by severity for each day after the first study vaccination (Days 1-8 post vaccination) and as the maximum severity over all 8 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the number and percentage of subjects reporting each symptom, any upper respiratory symptom and any systemic symptom by treatment arm.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). The number of SAEs, AESIs, and NOCMCs is expected to be small in this trial and will be reported by detailed listing showing the event description, MedDRA preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. If more than 5% of subjects report SAEs, and AESIs, and NOCMCs then the number and percentage of subjects reporting each type of event will be reported by treatment arm and compared using Fisher's Exact test. Unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA categories will be computed.

11.4.3. Secondary Immunogenicity Data

Summaries and analysis of secondary immunogenicity data will be presented for the mITT and PP populations.

Immune responses in terms of strain-specific (A/Brisbane/10/2007-like H3N2 (H3N2 M2SR) present in QIV, HAI and neutralization antibody titers will be summarized by treatment arm at each time point. Analyses will include number and proportion of subjects with a titer ≥1:40, number and proportion of subjects achieving seroconversion (defined as either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40 or a pre-vaccination titer ≥1:10 and a minimum four-fold rise in post-vaccination y titer), Geometric Mean Titers (GMTs) and Geometric Mean Fold Rise (GMFR) along with corresponding 95% confidence intervals (CIs).

Strain specific nasal sIgA responses with and without normalization to total sIgA at baseline will be summarized as the GMT and GMFR with corresponding 95% CIs. Descriptive summaries of the frequency of influenza H3 HA-specific (H3 M2SR-like and QIV-like viruses) and conserved internal viral protein-specific IFN-gamma ELISPOT will be provided.

Plots such as reverse cumulative distributions or longitudinal presentations of GMTs will be presented. Regression models of immune response controlling for baseline titers may be considered.

11.4.4. Exploratory Immunogenicity Data

Exploratory studies will be performed contingent on the results of the secondary immunogenicity analysis as noted in Section 3.2; additional details will be provided in the statistical analysis plan.

Summaries and analysis of exploratory immunogenicity data will be descriptive, including summary statistics and 95% CIs for each assay at each time point. Correlations between HAI and exploratory immune response outcomes will be evaluated. Graphical presentations may include plots such as reverse cumulative distributions or longitudinal presentations of GMTs will be presented.

11.4.5. Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

12. SOURCE DOCUMENTS ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical trial records for the purposes of quality assurance reviews, audits, evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, copies of transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

13. QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating site(s) and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site for clarification and resolution.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The site principal investigator (PI) will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979])) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The PI will also ensure conformity with ICH E6 Good Clinical Practice, and applicable federal regulations, guidance, and guidelines for Good Clinical Practice and Clinical Trials with humans.

14.2. Institutional Review Board

Each site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID before initiating recruitment of subjects. 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 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 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, and may cease if annual review is no longer required by applicable regulations. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

The IRB/IEC will determine that adequate provisions are made for soliciting the permission of each child's parent(s) or legal guardian, including whether permission of one parent is sufficient for research or whether permission is to be obtained from both parents. The IRB/IEC will determine how consent from subjects will be obtained when participating in the study is ongoing, and the subject has reached the age of majority.

14.3. 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 from the subject, parent(s)/legal guardian(s) of the minor subject. Subjects, parent(s)/legal guardian(s) will received a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential face to face with the subject, parent(s)/legal guardian(s). The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject, parent(s)/legal guardian(s).

Subjects, parent(s)/legal guardian(s) will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these alternative procedures.

Subjects, parent(s)/legal guardian(s) will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness for the subject to continue participating in the trial. Subjects, parent(s)/legal guardian(s) will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they will consist of, or where further information may be obtained. Subjects, parent(s)/legal guardian(s) 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 subject, parent(s)/legal guardian(s)

will be informed that the subject's participation is voluntary and 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.

The extent of the confidentiality of the subjects' records will be defined, and parent(s)/legal guardian(s) will be informed that applicable data protection legislation will be followed. Subjects, parent(s)/legal guardian(s) will be informed that the monitor(s), auditor(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, parent(s)/legal guardian(s) is authorizing such access.

Subjects, parent(s)/legal guardian(s) will be informed that records identifying the subject will e 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, parent(s)/legal guardian(s) 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, parent(s)/legal guardian(s) will be allowed sufficient time to consider their minor child's participating in this research trial, and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about the process prior to agreeing to participate.

Informed consent forms will be IRB-approved and parent(s)/legal guardian(s) will be asked to read and review the consent form. Subjects, parent(s)/legal guardian(s) must sign the informed consent form prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the informed consent form will be given to the parent(s)/legal guardian(s) for their records. The subjects, parent(s)/legal guardian(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site Clinical staff may pre-screen via chart review and refer

potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects, parent(s)/legal guardian(s) who consent to their minor child to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subject, parent(s)/legal guardian(s) will be re-consented per IRB requirements, if necessary. Subjects, parent(s)/legal guardian(s) will be given a copy of all informed consent forms that they sign.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Investigators will follow IRB/IEC requirements for enrollment of minors in this study. Minors will be informed about the study to the extent understandable to the minor. Investigators or designee will conduct the consent process with the parent(s)/legal guardian, who will be given an IRB/IEC-approved permission form, which may be referred to as a consent form, to read, review, and sign prior to any study procedures. The parent(s)/legal guardian will be provided meaningful study information including a statement that this study involves research, the child may not benefit from the trial, and the study involves risk. The required elements will be clearly presented, including the purpose of the study, the experimental procedures, the potential risks and discomforts, known adverse effects, possible benefits of the study for the subject, alternative therapies that may be beneficial, use and disclosure of private information, and other elements that are part of obtaining proper consent. The subject's parent(s)/legal guardian will be allowed sufficient time to discuss questions with the investigator.

The investigator or designee will describe in simplified terms the details of the study intervention/product, study procedures, risks and discomforts, benefits, and other consent elements, as appropriate. A separate IRB/IEC-approved assent form will be used for the minor, who may read and sign the form, or have it read to him/her prior to participating in study procedures. Assent may be obtained verbally or waived when approved by the IRB/IEC as appropriate to age. If a child declines to participate in the trial when assent is required by the IRB/IEC, the subject will not be enrolled even though the parents have provided permission. To ensure that consent is an ongoing process throughout the subject's participation in the study, the investigator and staff will review information as needed with the subject and the parent(s)/legal guardian and confirm that assent and permission are continuing. The permission and assent documents will be updated when new information is acquired that may impact the decision to continue in the study, and the subject's assent and the parent(s)/legal guardian's permission is obtained, as applicable.

The subject who reaches the age of majority will be consented at the next visit prior to study procedures. When no further visits are planned but the subject's participating is ongoing, the consent will be obtained via IRB/IEC-approved processes.

A minor's signature is not required on the assent form. Parental consent will be documented on the ICF document. In addition, the parent/legal guardian will sign under the assent section of the ICF that their child appears to understand the research to the best of their ability and agreed to participate.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all children and adolescents ages 9-17 years who meet the Subject Inclusion Criteria (see Section 5.1) and do not meet the Subject Exclusion Criteria (see Section 5.2), regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study.

No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor or other authorized representatives of the sponsor or governmental 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 subjects in this study. The clinical study site will permit access to such records. All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the

researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States 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 Federal Food and Drug Administration (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.

14.6 Study Discontinuation

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC. If the subject's private information will continue to be collected for this study, the IRB/IEC must approve a consent form with the study procedures, any risks and discomforts, and applicable elements, and the investigator or designee will re-consent the subjects as approved by the IRB/IEC.

14.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, 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 trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this trial.

14.8 Future Use of Stored Specimens and Data

Residual samples/specimens are those that are left over after the study has been completed. Parent(s)/legal guardian(s) of minor subjects must give consent to keep any remaining (residual) specimens (serum, PBMCs, plasma) derived from venous blood samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These residual clinical samples will be stored indefinitely at a central clinical storage facility and may be shared with investigators at the participating VTEU site and with other investigators at other institutions once the clinical study report has been finalized. The recipients of specimens will be informed that these specimens have a NIH certificate of confidentiality. An IRB will review future research prior to the use of identifiable specimens or data.

Parent(s)/legal guardian(s) of minor subjects must give consent to keep samples/specimens collected into a PAXgene tube during the study specifically for future use, including but not limited to future research studies that may test for other immune responses such as natural or antibodies from the vaccine to act against other bacterial or viruses. The future research clinical samples will be stored indefinitely at a central storage facility.

Residual specimens will be available upon the completion of this trial. Extra samples/specimens collected during the study may be requested from DMID and shipped from the DMID CMS while this study is ongoing or after the study, after IRB review.

There are no benefits to subjects in the collection, storage and subsequent future use of their samples/specimens. Future research tests may benefit others by leading to new approaches in the development of vaccines or treatments for influenza. Future use samples/specimens will not be sold or used directly for production of any commercial product. Each sample/ specimen will be encoded (labeled) only with a barcode and a unique tracking number that connects to a code key at the study site. Restricted access to the code key is maintained by the PI to protect subject confidentiality.

Reports from future research studies performed using subjects' samples/specimens will NOT be kept in their health records and results will not be shared with subjects.

For 19 subjects enrolled in the 2018-2019 influenza season, PAXgene tubes were obtained. These samples/specimens might be used for genetic testing, in new or different immunological laboratory tests, to provide information for the development of new vaccines, or for the studies of influenza or other infections. Parent(s)/legal guardian(s) of minor subjects will be contacted for re-consent for genetic testing. If the subject reached the age of 18, he/she will be contacted to give consent for genetic testing, as well as re-consent for all future research, as they have reached the age of adulthood.

After all subjects had enrolled, it was determined that genetic testing may be performed on residual specimens and samples/specimens collected during this trial specifically for the purpose of future use, under a secondary research protocol. Secondary research is research that is not part of this trial and will be performed in the future. Subjects will be informed and asked for their consent to use their residual specimens and samples/specimens collected during this trial specifically for the purpose of future research and information for genetic testing for “secondary research”. Genetic testing could include transcriptomics, whole genome or exome sequencing, or other types of genetic testing. This could contribute to identifying genetic factors involved in vaccine responses. Genetic testing results (data) may be shared broadly with other researchers but will not be shared with subjects. Individual results will be stored indefinitely in an NIH restricted-access database, and a summary of results may be placed in an unrestricted (open) database.

The samples/specimens will be labeled as above for storage. The genetic data in the NIH database will be coded in such a way that information will be considered de-identified. Subjects will be informed of the risks, including potential for re-identification and possible harm from the mis-use of data, however, NIH will require researchers to not re-identify the subjects.

Subjects will have the right to withdraw consent for the use of their samples/specimens and/or data without penalty at any time. Samples/specimens and data that have already been used for secondary research may not be able to be withdrawn.

"Written", or "signed", refers to the use of or writing on a paper or an electronic ICF. Re-consent (revised or separate consent form) for secondary research using samples/specimens and information, such as for genetic testing, may use paper or electronic ICFs to obtain the subject’s written consent. The subject will have a discussion with study staff, who is able to answer the subject’s questions, including if re-consent is obtained off-site. A paper copy of the signed ICF will be given to the subject in person or mailed.

14.9 Disclosure of Study Related Information

Parent(s)/legal guardian(s) can choose to receive written disclosure of their minor subject's treatment assignment after the database has been locked.

15. DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Blue or black permanent ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

15.1. Data Management Responsibilities

Data collection is the responsibility of the study personnel at each participating VTEU site under the supervision of the respective site principal investigator. During this trial, the site principal investigator must maintain complete and accurate documentation for this trial.

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2. Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments, and clinical laboratory values), and reactogenicity will be collected on data collection forms by study personnel than entered into a eCRF via a 21 CFR 11-compliant internet data entry system provided by the study data coordinating center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

15.3. Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

15.4. Timing/Reports

An interim report of blinded MN results will be provided after the 19 subjects enrolled in 2018 have completed the Day 113 visit. This streamlined report will be made available to the sponsor and vaccine partner for planning subsequent trials. These analyses will not be used to make any decisions concerning the conduct of this trial.

Additional safety data may be requested by the vaccine partner during study follow-up for the purpose of planning future trials. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Reports may include data summarized by unblinded treatment arm; if so, the presentation will be in a format that prevents inadvertent unblinding of any individual subject. Such reports will be made available to the sponsor and vaccine partner for planning subsequent trials. These analyses will not be used to make any decisions concerning the conduct of this trial. No hypothesis testing will be included in any interim report.

Once the last subject completes the last immunogenicity outcome visit (approximately 21 days after the second study vaccination), all serum samples will be sent for secondary immunogenicity assays (HAI, MN, and sIgA). After such data are reported in the database, and the data are considered clean and complete, a “topline” subset of the immunogenicity tables planned for the CSR will be provided to DMID on an expedited timeline.

Analysis of exploratory immunogenicity endpoints may be performed and released as the data are available from the research laboratory. Any such analyses would be considered the final analysis for the endpoint, and included in the CSR. The final CSR will be completed after the last subject’s last visit is completed, and the final clinical database including all long-term safety follow-up data is cleaned, monitored and locked and all secondary immunogenicity endpoint data are received. Additional exploratory immunogenicity endpoint data not available at the time of CSR preparation may be included in an addendum to the CSR.

Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and immunogenicity summary reports may be generated for the SMC.

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the participating VTEU site with a summary of results by treatment arm and/or subject treatment assignments. In this regard, the participating VTEU site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5. Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there is no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator 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 per the SDCC protocol deviation reporting procedures.

All deviations, as defined above, must be addressed in study subject 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. Protocol deviations must be sent to the local IRB per its

guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

16. PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClinicalTrials.gov.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11

NIH NOT-OD-16-149

17. LITERATURE REFERENCES

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APPENDIX A : SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**Early Term**	**Unsched-uled**
Study Day post first study vaccination	**Screen -28 to -1**	**Dose 1* D1**	**D8 ±1**	**D22 ±2**	**D57 ±3**	**Dose 2* D92 ±14**	**D 113**	**D 240**	**D 366**		
Study Day post second study vaccination						**D1**	**D22 ±2**	**D149 ±14**	**D275 ±14**		
Obtain Informed Consent/Assent	X										
Collect Demographic Information	X										
Review Eligibility Criteria	X	X				X					
Medical History	X	X	X[1]	X[1]	X[1]	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X[2]	X[2]	X[2]			X[#3]	X[#3]
Counsel about avoidance of taking aspirin and salicylate containing products	X	X	X	X	X	X	X			X	X
Oral Temperature		X				X				X	X

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**		
Study Day post first study vaccination	**Screen -28 to -1**	**Dose 1* D1**	**D8 ±1**	**D22 ±2**	**D57 ±3**	**Dose 2* D92 ±14**	**D 113**	**D 240**	**D 366**	**Early Term**	**Unsched-uled**
Study Day post second study vaccination						**D1**	**D22 ±2**	**D149 ±14**	**D275 ±14**		
Physical Examination	X										
Limited Physical Examination		X	X							X@	X@
Age and developmentally appropriate counseling about pregnancy prevention	X	X									
Targeted Physical Exam			{X}	{X}	{X}	{X}	{X}			X	X
Urine Pregnancy Test	X^	X^				X^					

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**Early Term**	**Unsched-uled**
Study Day post first study vaccination	**Screen -28 to -1**	**Dose 1* D1**	**D8 ±1**	**D22 ±2**	**D57 ±3**	**Dose 2* D92 ±14**	**D 113**	**D 240**	**D 366**		
Study Day post second study vaccination						**D1**	**D22 ±2**	**D149 ±14**	**D275 ±14**		
WBC, HGB, HCT, Platelet Count, ALT, AST, Serum Creatinine, PT, and aPTT	X		X	X[4,5]	X[4,6]		X[4]				
Ferritin Level	X						X				
Serum (HAI, HA/NA ELISA, Neutralization Assays)		X	X	X	X	X	X			X	
Nasal Swab sIgA		X	X	X	X	X	X			X	
PBMC (IFN-γ ELISPOT and CFSE Dilution/ICS Assays)		X	X	X	X	X[7]	X[7]			X[7]	
Enrollment in AdvantageEDC[SM] and Randomization		X									
Pre-Administration Reactogenicity		X				X					

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**Early Term**	**Unsched-uled**
Study Day post first study vaccination	**Screen -28 to -1**	**Dose 1* D1**	**D8 ±1**	**D22 ±2**	**D57 ±3**	**Dose 2* D92 ±14**	**D 113**	**D 240**	**D 366**		
Study Day post second study vaccination						**D1**	**D22 ±2**	**D149 ±14**	**D275 ±14**		
Study Vaccination		X				X					
30-minute Evaluation After Study Vaccination		X				X					
Distribute Memory Aid and Study-Related Materials		X									
Review Memory Aid			X							X[@]	X[@]
AE Assessment		X	X	X						X[#]	X[#]
AESI + NOCMC Assessment			X	X	X	X				X[!]	X[!]
SAE Assessment		X	X	X	X	X	X	X	X	X	X
Phone Call								X	X		

{}Targeted physical examination if indicated based on review of complete or interim medical history
^Will be performed on all female adolescents of childbearing potential at screening and within 24 hours prior to each study vaccination and results must be negative and known prior to each study vaccination.
[#]If within 21 days post first study vaccination.
*On dose dates, samples must be collected pre-dose
[@]If within 8 days after 1st vaccination
[!]If within 3 months post first study vaccination
[1]Subject's parent(s)/legal guardian(s) will be asked if the subject had any additional blood taken for medical reasons.
subject's blood taken for medical reasons
[2]Concomitant medications will be limited to collecting aspirin and salicylate containing products
[3]Aspirin or salicylate containing products will be collected if within 30 days of the first vaccination through 21 days after the last vaccination
[4]Hemoglobin only
[5]Collect at Day 22 if Hgb results from screen to Day 8 drop ≥0.5 g/dL or < lower limits of normal
[6]If Hgb is not collected at Day 22 or low at Day 22, collect at Day 57
[7]Will not collect PBMCs if Day 57 HgB is low