A Phase II Study to Evaluate and Compare the Immunogenicity of Monovalent Inactivated Influenza A/H7N9 Virus Vaccine Administered with and without AS03 Adjuvant and Monovalent Inactivated Influenza A/H3N2v Virus Vaccine Administered without Adjuvant in Healthy Adults through Standard and Systems Biology Analyses

DMID Protocol Number: 14-0015

DMID Funding Mechanism: Vaccine and Treatment Evaluation Units

Pharmaceutical Support Provided by:

Sanofi Pasteur
GlaxoSmithKline Biologicals

IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Principal Investigator: C. Buddy Creech, MD, MPH

DMID Clinical Project Manager: Wendy Buchanan, BSN, MS

DMID Medical Monitor: Mohamed Elsafy, MD

DMID Medical Officer: Francisco Leyva, MD

DMID Scientific Lead: Chris Roberts, PhD

DMID Contracting Officer: Kathy Ormanoski, MS, CCRA

Version Number: 3.0

17 July 2019

STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonisation (ICH) E6; 62 Federal Register 25691 (1997);
- The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule-Final Modification (45 CFR Parts 160 and 164);
- National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protections Training.

SIGNATURE PAGE

The signature below constitutes the approval of the protocol and the attachments, and provides the necessary assurances that this study 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 guidelines.

Site Principal Investigator:

Signed: _____ Date: _____

C. Buddy Creech, MD, MPH
Associate Professor, Pediatric Infectious Diseases
Director, Vanderbilt Vaccine Research Program

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LIST OF ABBREVIATIONS

A/H1N1	Influenza A Virus of the H1N1 Subtype
A/H3N2v	Influenza A Virus of the H3N2 Variant Subtype
A/H5N1	Influenza A Virus of the H5N1 Subtype
A/H7N7	Influenza A Virus of the H7N7 Subtype
A/H7N9	Influenza A Virus of the H7N9 Subtype
AdvantageEDC^{SM}	Electronic Data Capture System
AE	Adverse Event/Adverse Experience
AESIs	Adverse Events of Special Interest
AS03	Adjuvant System (03)
α	Alpha
β	Beta
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body Mass Index
BP	Blood Pressure
BPM	Beats Per Minute
CAR	Clinical Agents Repository
CBA	Cytometric Bead Arrays
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CMI	Cell-Mediated Immunity
CSR	Clinical Study Report
°C	Degrees Celsius
°F	Degrees Fahrenheit
D	Day(s)
DC	Dendritic Cell
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic Case Report Form
ESR	Erythrocyte Sedimentation Rate
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FWA	Federalwide Assurance
GBS	Guillain-Barré Syndrome

GCP	Good Clinical Practice
GMT	Geometric Mean Titer
GSK	GlaxoSmithKline Biologicals
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HRSA	Health Resources and Services Administration
IATA	International Air Transport Association
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IFN	Interferon
IL	Interleukin
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
iTRAQ	Isobaric Tags for Relative and Absolute Quantitation
ITT	Intent-to-Treat
IUD	Intrauterine Device
MAAEs	Medically-Attended Adverse Events
MACS	Magnetic Cell Separation
mcg or µg	Microgram(s)
MedDRA®	Medical Dictionary for Regulatory Activities
mL	Milliliter(s)
mm	Millimeter(s)
mmHg	Millimeters of Mercury
MOP	Manual of Procedures
N	Number of Subjects
Neut	Neutralizing or Neutralization
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NK	Natural Killer
NIH	National Institutes of Health
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NOCMCs	New-Onset Chronic Medical Conditions
OHRP	Office for Human Research Protections
PBS	Phosphate-Buffered Saline
PHI	Protected Health Information
PK	Pharmacokinetics

PIMMCs	Potentially Immune-Mediated Medical Conditions
PMN	Polymorphonuclear Leukocyte
PP	Per Protocol
PREP Act	Public Readiness and Emergency Preparedness Act
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
SRID	Single Radial Immunodiffusion
TMT	Tandem Mass Tags
TNF	Tumor Necrosis Factor
US	United States
V	Visit(s)
VTEU	Vaccine and Treatment Evaluation Unit
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase II Study to Evaluate and Compare the Immunogenicity of Monovalent Inactivated Influenza A/H7N9 Virus Vaccine Administered with and without AS03 Adjuvant and Monovalent Inactivated Influenza A/H3N2v Virus Vaccine Administered without AS03 Adjuvant in Healthy Adults through Standard and Systems Biology Analyses
Phase:	II
Population:	Approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, in the US
Number of Sites:	One Vaccine and Treatment Evaluation Unit (VTEU) site: Vanderbilt University
Study Duration:	Approximately 36 months
Subject Participation Duration:	Approximately 14 months
Estimated Time to Complete Enrollment:	Approximately 6 months
Description of Agent or Intervention:	Two doses (3.75 micrograms [mcg] of hemagglutinin [HA]) of a monovalent inactivated influenza A/H7N9 virus vaccine (A/Shanghai/2/2013) manufactured by Sanofi Pasteur administered intramuscularly approximately 28 days apart given with and without AS03 adjuvant manufactured by GlaxoSmithKline Biologicals (GSK) One dose (15 mcg HA) of a monovalent inactivated influenza A/H3N2v virus vaccine (A/Minnesota/11/2010) manufactured by Sanofi Pasteur administered intramuscularly

Study Objectives and Outcome Measures:

Primary Objectives

1. To assess the serum anti-HA hemagglutination-inhibition (HAI) response to influenza A/H7N9 antigen (with and without adjuvant) at Day 57 (approximately one month after the second study vaccination with A/H7N9 vaccine ± AS03) and influenza A/H3N2v antigen at Day 29 (approximately one month after the study vaccination with A/H3N2v vaccine).

2. To identify differentially expressed genes in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9 vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03), compared to baseline assessments performed prior to each study vaccination (Days -7, 1, and 29).

Primary Outcome Measures

1. Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre- vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 28 days after the second study vaccination (A/H7N9 vaccine, with and without adjuvant).

2. Percentage of subjects achieving seroconversion at approximately 28 days after the study vaccination with A/H3N2v vaccine.

3. Number of differentially expressed genes based on RNA expression as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine, as compared to baseline assessments performed prior to vaccination (Days -7, 1, and 29).

Secondary Objectives

1. To compare plasma cytokine and chemokine profiles at specific time points and between treatment arms:
    a. Post-vaccination time points [(Days 2 and 4; Day 8 (A/H3N2v arm only)], and Days 30, 32, and 36 (A/H7N9 arms only) with pre-vaccination time points (Days -7 and 1, prior to first study vaccination and Day 29, prior to second study vaccination (A/H7N9 arms only).

2. To assess the neutralizing antibody responses to influenza A/H7N9 antigen (with and without adjuvant) at Day 57 (approximately one month after the second study vaccination with A/H7N9 vaccine ± AS03) and influenza A/H3N2v antigen at Day 29 (approximately one month after the study vaccination with A/H3N2v vaccine).

3. To identify differentially expressed genes in human immune cells on Days 2, 4, and 8 following one intramuscular dose of influenza A/H3N2v vaccine compared to baseline assessments performed prior to study vaccination (Day -7 and Day 1).

Secondary Outcome Measures

1. Plasma measurement of cytokines and chemokines at each study visit, comparing:
    a. Post-vaccination time points with pre-vaccination time points by treatment arms.
    b. AS03-adjuvanted influenza A/H7N9 responses to unadjuvanted influenza A/H7N9 responses.
    c. Influenza A/H3N2v vaccine to unadjuvanted influenza A/H7N9 vaccine.
    d. Influenza A/H3N2v vaccine to adjuvanted A/H7N9 vaccine.

2. Serologic response to influenza hemagglutinin:

a. Percentage of subjects achieving seroconversion (defined as either a pre-vaccination Neut titer <1:10 and a post-vaccination Neut titer ≥1:40 or a pre-vaccination Neut titer ≥1:10 and a minimum four-fold rise in post-vaccination Neut titer) at approximately 28 days after the second study vaccination (A/H7N9 vaccine, with and without adjuvant).
b. Percentage of subjects achieving seroconversion at approximately 28 days after the study vaccination with influenza A/H3N2v vaccine.
c. Geometric Mean Titers of serum HAI and Neut antibody at baseline (Day -7 and 1) and 28 days after influenza A/H3N2v vaccination.
d. Geometric Mean Titers of serum HAI and Neut antibody at baseline (Day -7 and 1), and 28 days after both the first and the second dose of influenza A/H7N9 vaccine (with and without adjuvant).

3. Number of differentially expressed genes based on RNA-Seq analysis in human immune cells at Days 2, 4, and 8 after study vaccination, as compared to baseline assessments (Days -7 and 1) after influenza A/H3N2v vaccine.

Tertiary Objectives

1. To assess the frequency of adverse events.

2. To identify differentially abundant cellular proteins in human immune cells on Days 2, 4, and 8 following one intramuscular dose of influenza A/H3N2v vaccine compared to baseline assessments performed prior to study vaccination (Day -7 and Day 1).

3. To identify differentially abundant cellular proteins in human immune cells on Days 2, 4, and 29 (following first study vaccination with A/H7N9 vaccine ± AS03) and Days 30, 32, and 36 (following second study vaccination with A/H7N9 vaccine ± AS03), compared to baseline

assessments performed prior to study vaccination (Days -7, 1, and 29).

Tertiary Outcome Measures

1. Occurrence of solicited local reactogenicity events within 7 days after each study vaccination.

2. Occurrence of solicited systemic reactogenicity events within 7 days after each study vaccination.

3. Occurrence of unsolicited AEs collected for approximately 28 days after last study vaccination.

4. Occurrence of SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions for approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after last study vaccination.

5. Number of differentially abundant proteins based on protein abundance as determined by quantitative proteomics in human immune cells at Days 2, 4, and 8 after study vaccination, as compared to baseline assessments (Days -7 and 1) after influenza A/H3N2v vaccine.

6. Number of differentially abundant proteins based on protein abundance as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in protein abundance between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

Exploratory Objectives

1. To identify and characterize differentially expressed genes in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9

vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03) that differ between treatments (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

2. To identify and characterize differentially abundant cellular proteins in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9 vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03) that differ between treatments (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

3. To identify and characterize differentially expressed genes in human immune cells on Days 2 and 4 (following the first study vaccination) that differ in baseline responses (Days -7, and 1) between treatments (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

4. To identify and characterize differentially abundant cellular proteins in human immune cells on Days 2 and 4 (following the first study vaccination) that differ in baseline responses (Days -7, and 1) between treatments (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

5. To identify transcriptomic and proteomic signatures in human immune cells that correlate with influenza seroconversion and with peak HAI titers.

6. To correlate plasma cytokine profiles with transcriptomic and proteomic profiles in subjects receiving influenza A/H3N2v vaccine or influenza A/H7N9 ± AS03 vaccine.

7. Exploratory inspection of transcriptomics\proteomics response signatures for subjects with unexpected or severe safety profiles that are related to the study products.

Exploratory Outcome Measures

1. RNA expression, clusters of co-expressed genes, enriched pathways, and other functional modules as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in RNA expression between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

2. Protein abundance, clusters of co-abundant proteins, enriched pathways, and other functional modules as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in protein abundance between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

3. RNA expression, clusters of co-expressed genes, enriched pathways, and other functional modules as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, and 29 after study vaccination comparing baseline changes in RNA expression between treatment arms (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

4. Protein abundance, clusters of co-abundant proteins, enriched pathways, and other functional modules as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, and 29 after first study vaccination comparing baseline changes (Days -7, and 1) in protein abundance between treatment arms (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

5. Changes from baseline (Days -7, 1, and 29) in RNA expression or protein abundance that best correlate with influenza A/H7N9 seroconversion and with peak HAI titers at Days 2 and 4 (following first study vaccination) as well as Days 30, 32, and 36 (following second study vaccination).

6. Changes from baseline (Days -7, 1, and 29) in RNA expression or protein abundance that best correlate with baseline changes in cytokine concentrations at Days 2, 4, 8 (A/H3N2v arm only) and 29 (following first study vaccination) as well as Days 30, 32, and 36 (following second study vaccination).

7. Exploratory summaries of transcriptomics/proteomics response signatures for subjects with unexpected or severe safety profiles.

8. Lists of differential genes and proteins identified as part of the primary and secondary objectives.

Description of Study Design:

This is a single center, randomized, partially-blinded, Phase II, small, targeted, prospective study in approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, designed to evaluate and compare the immunogenicity between an intramuscular monovalent inactivated influenza A/H7N9 virus vaccine manufactured by Sanofi Pasteur given with and without AS03 adjuvant manufactured by GlaxoSmithKline, and an intramuscular unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur.

This study will use a standard and systems biology approach to assess the human early gene and protein signatures expressed at baseline (approximately Days -7 and 1), and at approximately Days 2, 4, and 8 (A/H3N2v-vaccinated subjects only) after the first study vaccination in each treatment arm as well as at approximately Days 29, 30, 32, and 36 in A/H7N9-vaccinated

subjects only. Cellular immunogenicity (systems biology studies) data will be integrated with serologic immunogenicity (HAI and Neut antibody assays) and reactogenicity data to develop a systems model of the human immune response to unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine and monovalent inactivated influenza A/H7N9 virus vaccine given with and without AS03 adjuvant.

This study will use venous blood samples and subject data collected from a total of thirty vaccinated subjects randomly divided into three equal treatment arms. The first treatment arm (n=10) will be vaccinated with one dose of 15 µg of A/H3N2v HA. The second treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given with AS03 approximately 28 days apart. The third treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given without AS03 adjuvant approximately 28 days apart. All study vaccinations will be administered intramuscularly.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study vaccination through 8 days after each study vaccination. Unsolicited non-serious adverse events (AEs) will be collected from the time of each study vaccination through approximately 28 days after each study vaccination. Serious adverse events (SAEs) and medically-attended adverse events (MAAEs) including new-onset chronic medical conditions (NOCMCs) and potentially immune-mediated medical conditions (PIMMCs) will be collected from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after the last study vaccination.

Venous blood samples (approximately 90 mL) will be collected from subjects at approximately Days -7 and 1 (immediately prior to the first study vaccination), and at approximately Days 2, 4; and 8 (A/H3N2v-vaccinated subjects only) after the first study vaccination for systems biology studies (cytokine and chemokine levels, immune cell activation status, and whole transcriptome and proteome profiles of the major blood immune cells).

Additionally, subjects in either treatment arm receiving A/H7N9 vaccine will have additional blood samples collected at approximately Days 29 (this will be collected immediately prior to the second study vaccination for subjects in either group receiving A/H7N9), 30, 32, and 36 after the first study vaccination for systems biology studies.

Serological assessment (hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays) will also be conducted on venous blood samples (approximately 10 mL) collected from subjects at Day 1 (immediately prior to the first study vaccination), and at approximately Days 29 (for A/H7N9-vaccinated subjects this will be collected immediately prior to the second study vaccination) and 57 (A/H7N9-vaccinated subjects only) after the first study vaccination.

We will quantify and characterize serum cytokine/chemokine levels, as well as transcriptomic and proteomic profiles from individual immune cell compartments, comparing this with standard serologic assessment to vaccine.

Urine samples (~20 mL) will also be collected from subjects on Days -7, 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29, 30, 32, and 36 for future research. *Note: Days 29, 30, 32, and 36 will apply only to A/H7N9-vaccinated subjects.*

Overview of Study Design:

Objective	Study Day	Assays
Baseline pre-vaccination (1)	Day -7	RNA-seq/proteomics from Immune cell compartments (ICC) Urine samples
Baseline pre-vaccination (2) and First Study Vaccination	Day 1	RNA-seq/proteomics from ICC Urine samples HAI and Neut Assays
Early innate immune response to first study vaccination	Day 2	RNA-seq/proteomics from ICC Urine samples
Intermediate innate immune response to first study vaccination	Day 4	RNA-seq/proteomics from ICC Urine samples
Late innate immune response and early adaptive immune response to first study vaccination (A/H3N2v-vaccinated subjects only)	Day 8	RNA-seq/proteomics from ICC Urine samples
Mature adaptive immune response to first study vaccination and new baseline for Second Study Vaccination	Day 29	RNA-seq/proteomics from ICC (A/H7N9-vaccinated subjects only) Urine samples (A/H7N9 vaccinated-subjects only) HAI and Neut Assays
Early innate immune response to second study vaccination (A/H7N9-vaccinated subjects only)	Day 30	RNA-seq/proteomics from ICC Urine samples
Intermediate innate immune response to second study vaccination (A/H7N9-vaccinated subjects only)	Day 32	RNA-seq/proteomics from ICC Urine samples
Late innate immune response and early adaptive immune response to second study vaccination (A/H7N9-vaccinated subjects only)	Day 36	RNA-seq/proteomics from ICC Urine samples
Final serologic assessment (A/H7N9-vaccinated subjects only)	Day 57	HAI and Neut Assays

Treatment Arms:

Treatment Arms (n=10 vaccinated subjects per Treatment Arm)	First Study Vaccination (Day 1)	Second Study Vaccination (Day 29±2 days)
1	15 µg A/H3N2v*	N/A
2	3.75 µg A/H7N9 + AS03	3.75 µg A/H7N9 + AS03
3	3.75 µg A/H7N9*	3.75 µg A/H7N9*
Total N=30 vaccinated subjects		

* unadjuvanted

1 KEY ROLES

DMID Clinical Project Manager:

Wendy Buchanan, BSN, MS
Division of Microbiology and Infectious Diseases
NIAID, NIH
[redacted]

DMID Scientific Lead:

Chris Roberts, PhD
Division of Microbiology and Infectious Diseases
NIAID, NIH
[redacted]

DMID Medical Officer:

Francisco Leyva, MD
Division of Microbiology and Infectious Diseases
NIAID, NIH
[redacted]

DMID Medical Monitor:

Mohamed Elsafy, MD
Division of Microbiology and Infectious Diseases
NIAID, NIH
[redacted]

DMID Contracting Officer:

Kathy Ormanoski, MS, CCRA
Division of Microbiology and Infectious Diseases
NIAID, NIH
[redacted]

Principal Investigator:

C. Buddy Creech, MD, MPH
Associate Professor, Pediatric Infectious Diseases
Director, Vanderbilt Vaccine Research Program
Vanderbilt University School of Medicine
[redacted]

Co-Investigators:

Kathryn M. Edwards, MD
Sarah H. Sell and Cornelius Vanderbilt Chair in Pediatrics
Vanderbilt Vaccine Research Program
Vanderbilt University Medical Center
[redacted]

Isaac Thomsen, MD, MSCI
Assistant Professor, Pediatric Infectious Diseases
Vanderbilt University School of Medicine
[redacted]

Leigh Howard, MD, MPH
Assistant Professor, Pediatric Infectious Diseases
Vanderbilt University School of Medicine
[redacted]

James E. Crowe, Jr., MD
Ann Scott Carell Chair
Vanderbilt Vaccine Center
Vanderbilt University Medical Center
[redacted]

Institutions:	**Vanderbilt University Medical Center** Vanderbilt Vaccine Research Program [redacted]
Safety and Pharmacovigilance Contractor:	**DMID Pharmacovigilance Group** Clinical Research Operations and Management Support (CROMS) [redacted]
Statistical and Data Coordinating Center:	**The Emmes Corporation** [redacted]
Study Agent Repository:	**Fisher BioServices** c/o DMID Clinical Agents Repository Contract [redacted]
HAI and Neut Antibody Assays Laboratory:	**Southern Research** [redacted]

Systems Biology Studies Laboratories:

Vanderbilt Vaccine Research Program Laboratory

[redacted]

Vanderbilt Mass Spectrometry Proteomics Core Proteomics Laboratory

[redacted]

Hudson Alpha Institute for Biotechnology

[redacted]

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Since its emergence in Southeast Asia in 2013, influenza A/H7N9/Shanghai continues to infect humans and is associated with a high case fatality rate[1]. The virus holds significant pandemic potential, since the avian population is rarely symptomatic (making avian disease surveillance difficult), and there is little preexisting immunity to the virus in the human population[2]; however, its current inability to sustain efficient human-to-human transmission has limited its spread[3].

Unfortunately, as with other avian influenza strains, the immunogenicity of inactivated vaccines is poor. Higher doses of hemagglutinin (HA), a major target of the protective immune response, generate more frequent and higher antibody titers[4]; however clinical trials of influenza A/H5N1, influenza A/H7N7, and influenza A/H7N9 vaccines have clearly demonstrated that avian HA's are substantially less immunogenic than seen with seasonal vaccines, even when given in very large doses[5-7]. For example, in clinical studies of a subvirion inactivated influenza A/H5N1/Vietnam vaccine in healthy adults, two 90 mcg doses of vaccine were required to stimulate an antibody response in just over half (57%) of the recipients[8]. A NIAID sponsored Phase I/II trial studying the safety and immunogenicity of an inactivated influenza A/H7N9 vaccine revealed that even two doses of 90 mcg each were insufficient to generate measurable antibody responses in healthy adults[9]. Moreover, adjuvant was required to induce protective responses against inactivated A/H7N9 vaccine in two NIAID sponsored Phase I clinical trials. While 59% of subjects seroconverted when vaccinated with 3.75 mcg of A/H7N9 vaccine in the presence of MF59 adjuvant, only 5% of subjects seroconverted when vaccinated with 45 mcg of A/H7N9 vaccine in the absence of MF59 adjuvant[6,7].

The use of adjuvants is a promising alternative to increasing HA content or administering additional doses of vaccine. Adjuvants have the potential to increase both the cellular and humoral responses at a given dose of antigen, to decrease the amount of antigen needed in the vaccine, and to improve immunogenicity among those that generally respond poorly to influenza antigens (e.g., the elderly or immunocompromised). While the addition of adjuvants has sometimes been associated with increased injection site reactogenicity and occasionally systemic reactogenicity, the overall safety of novel oil-in water adjuvants such as MF59 (Novartis Vaccines and Diagnostics) and AS03 (GSK) is reassuring[10-14].

Currently, one AS03-adjuvanted influenza A/H5N1 vaccine and one MF59-adjuvanted influenza A/H5N1 vaccine (FLUAD) are licensed in the US, and both adjuvants are licensed in Europe as part of multiple influenza vaccines. When used as a component of influenza A/H5N1 vaccine,

AS03 leads to substantially improved HA responses when compared to non-adjuvanted vaccine[15-21]. In an early study, two doses of A/H5N1 vaccine, with and without AS03 were given, with dosages ranging from 3.75 to 30 mcg of HA. After two doses, only the 30 mcg non-adjuvanted formulation met the Committee for Medicinal Products for Human Use (CHMP) criteria for seroconversion (>40%) while CHMP criteria were met by all adjuvanted formulations, even down to 3.75 mcg of HA. Moreover, seroconversion criteria were met by HA doses of 7.5 mcg or greater in the adjuvanted group after only the first dose. These data, as well as data from the 2009 pandemic A/H1N1 experience, demonstrate the potency and safety of AS03 as an influenza vaccine adjuvant.

2.1.1 Public Readiness and Emergency Preparedness Act

The protocol and the study products (monovalent inactivated influenza A/H7N9 virus vaccine and monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur as well as AS03 adjuvant manufactured by GlaxoSmithKline Biologicals) tested are covered under the Public Readiness and Emergency Preparedness Act (PREP Act). Under the PREP Act, covered persons are immune from liability actions brought from the administration or use of a covered countermeasure that is the subject of a declaration. The PREP Act provides immunity for covered persons (such as manufacturers, distributers, program planners and other qualified persons who prescribe, administer or dispense the study vaccines with or without AS03 adjuvant) from tort liability, unless the injury was caused by willful misconduct.

The PREP Act also authorized a "Covered Countermeasures Process Fund" to provide compensation to eligible individuals who suffer specified injuries from administration or use of a countermeasure pursuant to the declaration. Any requests for compensation must be filed within one year of administration or use of the countermeasure. Requests would go to the Health Resources and Services Administration (HRSA) Preparedness Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/). Compensation may then be available for medical benefits, lost wages and death benefits to eligible individuals for specified injuries in accordance with regulations published by the Secretary of HRSA. Eligibility for compensation and the injuries for which compensation may be available are further defined by regulation.

An individual who suffers a serious physical injury or death from administration and use of the study vaccines with or without AS03 adjuvant must first seek compensation from the Covered Countermeasures Process Fund. A serious physical injury means an injury that is life threatening, results in, or requires medical or surgical intervention to prevent, permanent impairment of body function or permanent damage to body structure. Any compensation will be reduced by public or private insurance or worker's compensation available to the injured individual.

If no funds have been appropriated to the compensation program, the Secretary of HRSA does not make a final determination on the individual's request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the US District Court for the District of Columbia, but only if the claim involves willful misconduct, is pled with particularity required under the PREP Act, verified, and accompanied by an affidavit by a physician who did not treat the individual and certified medical records. Any award is reduced by any public or private insurance or worker's compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering, physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a United States Federal or a State court.

2.2 Scientific Rationale

The cellular and molecular mechanisms of action by which novel oil-in-water emulsion adjuvants enhance the immune response to vaccination are not completely understood[22,23]. Recently published data from mouse models suggest that AS03 works locally to stimulate immune effectors[24]. AS03 also induces the local production of monocyte-, dendritic cell (DC)-, and neutrophil-recruiting chemokines in mice, as well as the pro-inflammatory cytokines interleukin (IL)-6, IL-1β, IL-1α, tumor necrosis factor (TNF), and interferon (IFN)-gamma, and promotes recruitment of antigen-loaded monocytes to draining lymph nodes. AS03 is unique among adjuvants in that it includes α- tocopherol, a highly bio-available form of Vitamin E. The presence of α-tocopherol appears to be necessary to induce local cytokine responses and stimulate antigen-specific adaptive responses. The mechanism of action in humans is not well defined. Elucidating the mechanisms by which AS03 augments the immune response to influenza vaccine in humans is important not only in developing more efficient vaccine regimens, but also in determining the safety and the potential for adverse events after adjuvanted vaccine.

Recently, an increased incidence of narcolepsy was noted in patients aged 4-19 after receiving Pandemrix™, a monovalent H1N1 vaccine adjuvanted with AS03 licensed for use in Europe during the 2009-2010 influenza season[25-33]. In Finland, the risk of developing narcolepsy was nine times higher in patients aged 4-19 years who had received the pandemic vaccine than in those who had not. All of these patients had the human leukocyte antigen (HLA) DQB1*0602 genotype, a genotype which has been strongly linked to narcolepsy[34]. The pathophysiology of narcolepsy has been postulated to result from low levels of the hypothalamic peptide hypocretin (also known as orexin) in the central nervous system, which is the result of selective cell death of hypocretin-containing neurons. Many have hypothesized an immune-mediated phenomenon is responsible for hypocretin cell death since the disease is tightly associated with HLA haplotypes.

A recent report from Ahmed et al suggests that antibodies to influenza nucleoprotein cross-react with human hypocretin receptor 2[35]. The authors postulate that differences in vaccine NP content may explain the previously appreciated narcolepsy association with Pandemrix™, since NP content is higher than in some other influenza vaccines and the addition of AS03 may enhance the immune response to NP.

This study will use a standard and systems biology approach to assess the human early gene and protein signatures expressed in healthy adults before and after each of two doses of a monovalent inactivated influenza A/H7N9 virus vaccine manufactured by Sanofi Pasteur administered intramuscularly approximately 28 days apart at 3.75 mcg of HA (A/Shanghai/2/2013) per 0.5 mL [adjuvanted] or 0.25 mL [unadjuvanted] dose given with or without AS03 adjuvant manufactured by GSK. We will also compare these responses to one dose of unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur administered intramuscularly at 15 mcg of HA (A/Minnesota/11/2010) per 0.5 mL dose. The addition of an interpandemic (seasonal) strain of influenza A/H3N2v may allow us to compare the innate and adaptive immune response between avian and seasonal influenza vaccines as well.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of this study are those associated with having blood drawn, intramuscular (IM) injection of and possible reactions to the monovalent inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant and monovalent inactivated influenza A/H3N2v virus vaccine as well as breach of confidentiality.

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 may also cause transient discomfort and fainting. Drawing blood and 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.

The monovalent inactivated influenza A/H7N9 virus vaccine to be used in this study has never been tested for safety in animals. However, the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID) is sponsoring three ongoing Phase II clinical trials: two randomized, double-blinded, controlled clinical trials in healthy adults, 19 to 64 years old, inclusive, to assess the safety, reactogenicity, and

immunogenicity following receipt of two intramuscular doses, approximately 21 days apart, of a monovalent inactivated influenza A/H7N9 virus vaccine (produced by Sanofi Pasteur, Swiftwater, PA under contract to DHHS) administered admixed with or without adjuvants MF59 (Novartis) or AS03 (GlaxoSmithKline) (DMID Protocol 13-0032; NCT01938742[6] and DMID Protocol 13-0033; NCT01942265[7]); and one randomized, partially-blinded, controlled clinical trial in healthy adults, aged 65 years and older, to assess the safety, reactogenicity, and immunogenicity following receipt of three intramuscular doses of a monovalent inactivated influenza A/H7N9 virus vaccine (produced by Sanofi Pasteur, Swiftwater, PA under contract to DHHS) administered admixed with MF59 adjuvant (Novartis) at different intervals and dosages (DMID Protocol 13-0034; NCT02213354).

For DMID Protocol 13-0032 and DMID Protocol 13-0033, the dosages of the inactivated influenza A/H7N9 vaccine evaluated were: 3.75, 7.5, 15, and 45 mcg of HA per dose. Enrollment in both clinical trials is complete with a total of 700 (DMID Protocol 13-0032) and 980 (DMID Protocol 13-0033) study subjects enrolled, all doses of study products were administered, and safety follow-up of study subjects is complete. Overall, the study products administered in both clinical trials were generally safe and well-tolerated. For DMID Protocol 13-0032, nine SAEs were reported. All were assessed as being not related to study product. Two Adverse Events of Special Interest (AESIs) were reported in this clinical trial: Hashimoto's disease (assessed as not related to study product) and Hashimoto's thyroiditis (assessed as related to study product). For DMID Protocol 13-0033, sixteen SAEs were reported. Fifteen of these SAEs were assessed as being not related to study product. One SAE was considered to be related: acute inferior myocardial infarction. Two AESIs were reported in this clinical trial: psoriasiform dermatitis (assessed as not related to study product) and celiac disease (assessed as not related to study product).

The manufacturing process used to produce the monovalent inactivated influenza A/H7N9 virus vaccine to be used in this study is based on Sanofi Pasteur's current process for the production of their inactivated licensed seasonal influenza virus vaccine Fluzone®, except for a minor modification in the Phosphate-Buffered Saline (PBS) diluent in the formulation step that was made according to previous experiences of manufacturing of monovalent pandemic vaccines. This monovalent inactivated split influenza virus vaccine was derived from the influenza A/Shanghai/2/2013 virus. Licensed release testing specifications were maintained, with a modification to the potency test. [redacted]

We plan to use a 3.75 mcg of HA (A/Shanghai/2/2013) per 0.5 mL [adjuvanted] or 0.25 mL [unadjuvanted] dose in this study. Consequently, the potential risks to subjects are anticipated to be similar to those observed for Sanofi Pasteur's unadjuvanted licensed inter-pandemic (seasonal) influenza virus vaccines (Fluzone® and Fluzone® High-Dose), and their unadjuvanted licensed 2009 A/H1N1 and A/H5N1 monovalent vaccines.

The manufacturing process used to produce the monovalent inactivated influenza A/H3N2v virus vaccine to be used in this study is based on Sanofi Pasteur's current process for the production of their inactivated licensed seasonal influenza virus vaccine Fluzone®, except for a minor modification in the PBS diluent in the formulation step that was made to accommodate the nature of the diluted virus. This monovalent inactivated split influenza virus vaccine was derived from the influenza A/Minnesota/11/2010 virus. Consequently, the potential risks to subjects are anticipated to be similar to those observed for Sanofi Pasteur's unadjuvanted licensed inter-pandemic (seasonal) influenza virus vaccines (Fluzone® and Fluzone® High-Dose), and their unadjuvanted licensed 2009 A/H1N1 and A/H5N1 monovalent vaccines. The Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID) sponsored a Phase II open-label study in healthy adult and elderly populations to assess the safety, reactogenicity, and immunogenicity of the unadjuvanted A/H3N2v vaccine following receipt of two intramuscular doses (15mcg of HA/0.5mL) administered approximately 21 days apart (DMID Protocol 12-0011; NCT01746082[41]). Overall, the unadjuvanted A/H3N2v vaccine was generally safe and well-tolerated among the 210 vaccinated subjects. Ten SAEs were reported. All were assessed as being not related to study product.

Occasionally, adult recipients of unadjuvanted licensed, inactivated influenza virus vaccines 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 for pandemic vaccines based on those 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.

Acute and potentially life-threatening allergic reactions (i.e., anaphylaxis) are also possible. These reactions occur in about 1 in 4 million people given a vaccination. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or decrease in blood pressure (hypotension). If these reactions occur, then 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 death, although researchers do not expect this to occur.

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 virus 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[42]. Interestingly, although vaccination rates have increased in the last 10 years, the numbers of reported cases of vaccine-associated GBS have declined[43]. 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[44]. 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[45-47]. 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)[48]. An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS[49]. It is unknown if the administration of the monovalent inactivated influenza A/H7N9 virus vaccine or monovalent inactivated influenza A/H3N2v virus vaccine to be used in this study will result in an increased incidence of GBS as the mechanism leading to this adverse event has not been completely elucidated.

As of November 22, 2015 (per the most current version of the manufacturer's IB), data are available for 56 GSK-sponsored clinical trials of AS03-adjuvanted monovalent pandemic vaccines manufactured by GSK. More than 18,000 adult (age ≥18 years) and 6,900 pediatric (6 months to 17 years old) clinical trial participants have received at least one dose of a GSK-manufactured, AS03-adjuvanted monovalent pandemic influenza vaccine. Clinical data collected by GSK as of November 22, 2015 (per the most current version of the manufacturer's IB), suggest that inactivated monovalent (pre)pandemic influenza virus antigens adjuvanted with AS03 have generally acceptable safety and benefit/risk profiles, though the incidence rates of solicited local and systemic adverse events are higher with AS03-adjuvanted antigens than with antigen alone, a licensed trivalent influenza vaccine, or placebo. Some unsolicited adverse events (e.g., insomnia, dizziness, cystitis) were associated with a higher relative risk among H5N1/AS03 recipients in contrast to Fluarix® or placebo recipients.

The information and guidance that follow are based on pre-clinical and clinical study results for GSK-manufactured AS03-adjuvanted monovalent pandemic vaccines, post- marketing safety surveillance data seen with unadjuvanted, inactivated trivalent seasonal influenza vaccines and (in the case of the H1N1 vaccines) post-marketing safety surveillance data seen to date for both Pandemrix™ and Arepanrix™ H1N1 vaccines.

The reactogenicity profile in humans of GSK-manufactured AS03-adjuvanted vaccines is primarily conditioned by the presence of the adjuvant. The incidence and severity of local redness, swelling, and pain at the injection site in recipients of AS03-adjuvanted vaccines are increased relative to monovalent pandemic influenza antigen alone, a licensed trivalent influenza vaccine, or placebo. There is no increase in local and systemic reactogenicity events in recipients of AS03-adjuvanted vaccines after a second dose of vaccine relative to the first when given 21 days apart. In young children (6 months to 6 years old), increased frequency of fever has been observed following a heterologous booster dose of adjuvanted vaccine administered 6 months after the primary series.

As of November 22, 2015 (per the most current version of the manufacturer's IB), there has been no evidence collected by GSK in clinical trials to support a conclusion that any potential immune-mediated disease or group of diseases was causally related to an AS03-adjuvanted vaccine. There

have been no deaths in GSK clinical trials of AS03-adjuvanted pandemic influenza vaccines assessed as related to study vaccine. A total of 1,428 non-fatal SAEs have been reported for adult subjects as of November 22, 2015. Fifteen of these events were deemed related to vaccination by the Investigator or GSK. Of these, six occurred in recipients of an adjuvanted H1N1 vaccine: asthma, herpes zoster, hepatic enzyme increased, pain in extremity, polymyalgia rheumatic, and thrombocytopenia. Three occurred in recipients of unadjuvanted H1N1 vaccine: alanine aminotransferase increased, hypersensitivity, and multiple sclerosis. One SAE classified as related (myalgia) occurred in a subject who received a control product. Four SAEs classified as related occurred in recipients of an adjuvanted H5N1 vaccine: autoimmune hepatitis, angina pectoris, pulmonary embolism, and non-Hodgkin's lymphoma. One SAE classified as related (lobar pneumonia) occurred in a recipient of an unadjuvanted H5N1 vaccine. Overall, the reactogenicity and safety profile of AS03-adjuvanted pandemic vaccines is acceptable and no safety concerns have been identified in clinical trials.

Narcolepsy is a chronic sleep disorder with a background incidence rate, based on US data, of approximately 1.37 per 100,000 per year, with a peak onset between 10 and 19 years of age in some datasets. Narcolepsy, when associated with cataplexy is seen almost exclusively in individuals who are HLA DQB1*0602 allele carriers. An autoimmune etiology has been proposed. In the post-marketing period for adjuvanted H1N1 pandemic vaccines, several epidemiological studies conducted in several countries independently of GSK reported an increased risk of narcolepsy with or without cataplexy in subjects who were vaccinated with Dresden-manufactured H1N1 (Pandemrix™ H1N1) vaccine during the 2009-2010 season. These studies have described an absolute risk increase of narcolepsy of approximately 1.4 to 8 additional cases per 100,000 vaccinated children/adolescents, and approximately one additional case per 100,000 vaccinated adults compared to background rates of 0.12 to 0.79 per 100,000 children/adolescents per year and 0.67 to 1.10 per 100,000 adults per year. The observed temporal association between narcolepsy and vaccination with Pandemrix™ H1N1 is not fully understood, and further research to evaluate the association between narcolepsy and Pandemrix™ H1N1, and other possible contributory factors to the development of narcolepsy during the 2009-2010 pandemic, such as genetic and environmental factors, is being conducted. A GSK-supported study was conducted in Quebec, Canada, to assess the risk of narcolepsy associated with Arepanrix™ H1N1, using various index dates, risk periods, observation periods, and epidemiological designs. Overall, GSK considers that there is no strong evidence of an association between Q-Pan-H1N1 and narcolepsy in Quebec.

No post-marketing data are available for AS03 administered in combination with any GSK-manufactured H5N1, H7N1, H7N9 or H9N2 antigen. However, millions of doses of GSK-manufactured H1N1 antigens, combined with AS03, were administered in the context of the 2009/10 pandemic response. In addition to the adverse reactions reported in clinical trials, the

following have been reported during post-marketing experience with Pandemrix™ (H1N1) and Arepanrix™ (H1N1):

- Immune system disorders
  - Rare: anaphylaxis, allergic reactions
- Nervous system disorders
  - Rare: febrile convulsions (in subjects below 20 years of age), somnolence**, Guillain-Barré syndrome*
    **Spontaneous reports of Guillain-Barré syndrome have been received following vaccination with Arepanrix™ (H1N1); however, a causal association between vaccination and Guillain-Barré syndrome has not been established. Data from a post-marketing epidemiological study in Canada indicate a small but significant increased relative risk of Guillain-Barré syndrome of 1.80 (95% CI, 1.63-4.62) in the 56-day period following vaccination with Arepanrix™ (H1N1, in persons 50 years of age and older). The number of GBS cases attributable to vaccination was approximately 2 per 1 million doses.*
    ***reported in patients with narcolepsy and as a temporary event following vaccination*
  - Very rare[1]: narcolepsy with or without cataplexy
    [1]Frequency based on estimated attributable risk from epidemiological studies in several European countries.
- Skin and subcutaneous tissue disorders
  - Rare: angioedema, generalized skin reactions, urticaria
- General disorders and administration site conditions
  - Rare: injection site reactions (such as inflammation, mass, ecchymosis)

From post-marketing surveillance with interpandemic (seasonal) trivalent vaccines, the following additional adverse events have been reported:

- Blood and lymphatic system disorders
  - Transient thrombocytopenia
- Nervous system disorders
  - Neuralgia, convulsions
  - Neurological disorders, such as encephalomyelitis, neuritis and Guillain-Barré syndrome
- Vascular disorders
  - Vasculitis with transient renal involvement

It is unknown if the monovalent inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant or unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine poses

any risks to an unborn child. As of November 22, 2015 (per the most current version of the manufacturer's IB), the available data for women who become pregnant during clinical trials of AS03-adjuvanted (pre) pandemic influenza vaccines do not suggest any causal relationship between adverse pregnancy outcomes and receipt of an AS03-adjuvanted vaccine. Women of childbearing potential who are not surgically sterile via tubal sterilization, bilateral oophorectomy or hysterectomy, or who are not postmenopausal for ≥1 year must agree to practice highly effective contraception that may include, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms that contain spermicide or with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 60 days after their last study vaccination. A highly effective method of contraception is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all women of childbearing potential will be required to have a negative urine or serum pregnancy test within 24 hours prior to each study vaccination. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

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 study will be allowed access to the PHI that is collected. Any publications from this study 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 Food and Drug Administration (FDA).

A description of this clinical study 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 inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant, and there is no prospect of benefit at this time. It is possible that vaccination using the monovalent inactivated influenza A/H7N9 virus vaccine with or with AS03 adjuvant will result in some protection against infection caused by the influenza A/H7N9 virus. Vaccination using the monovalent inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant may or may not provide protection against a serious infection with the influenza A/H7N9 virus, should the participant be exposed. The duration of any such protection is currently unknown. The monovalent inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant is not expected to offer protection against circulating seasonal influenza viruses. There may be pandemic preparedness benefits to society in the future if the monovalent inactivated influenza A/H7N9 virus vaccine and AS03 adjuvant being evaluated here prove to be sufficiently safe and immunogenic and can be employed if a need for widespread influenza A/H7N9 vaccination occurs.

Similarly, there are no known benefits attributable to receipt of the unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine, but there is a prospect of benefit. A/H3N2v has been seen in the United States as a variant of the seasonal influenza A/H3N2; the virus has been seen primarily in those with pig exposures. As there may be cross-protection against other A/H3N2 viruses, and since the future circulation of A/H3N2v is unknown, vaccination may provide some benefit.

3 STUDY OBJECTIVES AND OUTCOME MEASURES

Primary Objectives

1. To assess the serum anti-HA hemagglutination-inhibition (HAI) response to influenza A/H7N9 antigen (with and without adjuvant) at Day 57 (approximately one month after the second study vaccination with A/H7N9 vaccine ± AS03) and influenza A/H3N2v antigen at Day 29 (approximately one month after the study vaccination with A/H3N2v vaccine).

2. To identify differentially expressed genes in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9 vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03), compared to baseline assessments performed prior to each study vaccination (Days -7, 1, and 29).

Primary Outcome Measures

1. Percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <1:10 and a post-vaccination HAI titer ≥1:40 or a pre- vaccination HAI titer ≥1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer) at approximately 28 days after the second study vaccination (A/H7N9 vaccine, with and without adjuvant).

2. Percentage of subjects achieving seroconversion at approximately 28 days after the study vaccination with A/H3N2v vaccine.

3. Number of differentially expressed genes based on RNA expression as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine, as compared to baseline assessments performed prior to vaccination (Days -7, 1, and 29).

Secondary Objectives

1. To compare plasma cytokine and chemokine profiles at specific time points and between treatment arms:
    a. Post-vaccination time points [(Days 2 and 4; Day 8 (A/H3N2v arm only)], and Days 30, 32, and 36 (A/H7N9 arms only) with pre-vaccination time points (Days -7 and 1, prior to first study vaccination and Day 29, prior to second study vaccination (A/H7N9 arms only).

2. To assess the neutralizing antibody responses to influenza A/H7N9 antigen (with and without adjuvant) at Day 57 (approximately one month after the second study vaccination with A/H7N9 vaccine ± AS03) and influenza A/H3N2v antigen at Day 29 (approximately one month after the study vaccination with A/H3N2v vaccine).

3. To identify differentially expressed genes in human immune cells on Days 2, 4, and 8 following one intramuscular dose of influenza A/H3N2v vaccine compared to baseline assessments performed prior to study vaccination (Day -7 and Day 1).

Secondary Outcome Measures

1. Plasma measurement of cytokines and chemokines at each study visit, comparing:
   a. Post-vaccination time points with pre-vaccination time points by treatment arms.
   b. AS03-adjuvanted influenza A/H7N9 responses to unadjuvanted influenza A/H7N9 responses.
   c. Influenza A/H3N2v vaccine to unadjuvanted influenza A/H7N9 vaccine.
   d. Influenza A/H3N2v vaccine to adjuvanted A/H7N9 vaccine.

2. Serologic response to influenza hemagglutinin:
   a. Percentage of subjects achieving seroconversion (defined as either a pre-vaccination Neut titer <1:10 and a post-vaccination Neut titer ≥1:40 or a pre-vaccination Neut titer ≥1:10 and a minimum four-fold rise in post-vaccination Neut titer) at approximately 28 days after the second study vaccination (A/H7N9 vaccine, with and without adjuvant).
   b. Percentage of subjects achieving seroconversion at approximately 28 days after the study vaccination with influenza A/H3N2v vaccine.
   c. Geometric Mean Titers of serum HAI and Neut antibody at baseline (Day -7 and 1) and 28 days after influenza A/H3N2v vaccination.
   d. Geometric Mean Titers of serum HAI and Neut antibody at baseline (Day -7 and 1), and 28 days after both the first and the second dose of influenza A/H7N9 vaccine (with and without adjuvant).

3. Number of differentially expressed genes based on RNA-Seq analysis in human immune cells at Days 2, 4, and 8 after study vaccination, as compared to baseline assessments (Days -7 and 1) after influenza A/H3N2v vaccine.

Tertiary Objectives

1. To assess the frequency of adverse events.

2. To identify differentially abundant cellular proteins in human immune cells on Days 2, 4, and 8 following one intramuscular dose of influenza A/H3N2v vaccine compared to baseline assessments performed prior to study vaccination (Day -7 and Day 1).

3. To identify differentially abundant cellular proteins in human immune cells on Days 2, 4, and 29 (following first study vaccination with A/H7N9 vaccine ± AS03) and Days 30, 32, and 36 (following second study vaccination with A/H7N9 vaccine ± AS03), compared to baseline assessments performed prior to study vaccination (Days -7, 1, and 29).

Tertiary Outcome Measures

1. Occurrence of solicited local reactogenicity events within 7 days after each study vaccination.

2. Occurrence of solicited systemic reactogenicity events within 7 days after each study vaccination.

3. Occurrence of unsolicited AEs collected for approximately 28 days after last study vaccination.

4. Occurrence of SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions for approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after last study vaccination.

5. Number of differentially abundant proteins based on protein abundance as determined by quantitative proteomics in human immune cells at Days 2, 4, and 8 after study vaccination, as compared to baseline assessments (Days -7 and 1) after influenza A/H3N2v vaccine.

6. Number of differentially abundant proteins based on protein abundance as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in protein abundance between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

Exploratory Objectives

1. To identify and characterize differentially expressed genes in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9 vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03) that differ between treatments (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

2. To identify and characterize differentially abundant cellular proteins in human immune cells on Days 2, 4, and 29 (following the first study vaccination with A/H7N9 vaccine ± AS03) and on Days 30, 32, and 36 (following the second study vaccination with A/H7N9 vaccine ± AS03) that differ between treatments (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

3. To identify and characterize differentially expressed genes in human immune cells on Days 2 and 4 (following the first study vaccination) that differ in baseline responses (Days -7, and 1) between treatments (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

4. To identify and characterize differentially abundant cellular proteins in human immune cells on Days 2 and 4 (following the first study vaccination) that differ in baseline responses (Days -7, and 1) between treatments (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

5. To identify transcriptomic and proteomic signatures in human immune cells that correlate with influenza seroconversion and with peak HAI titers.

6. To correlate plasma cytokine profiles with transcriptomic and proteomic profiles in subjects receiving influenza A/H3N2v vaccine or influenza A/H7N9 ± AS03 vaccine.

7. Exploratory inspection of transcriptomics\proteomics response signatures for subjects with unexpected or severe safety profiles that are related to the study products.

Exploratory Outcome Measures

1. RNA expression, clusters of co-expressed genes, enriched pathways, and other functional modules as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in RNA expression between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

2. Protein abundance, clusters of co-abundant proteins, enriched pathways, and other functional modules as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, 29, 30, 32, and 36 after study vaccination with AS03-adjuvanted and unadjuvanted A/H7N9 vaccine comparing changes in protein abundance between treatment arms (A/H7N9 vaccine vs. A/H7N9 vaccine + AS03).

3. RNA expression, clusters of co-expressed genes, enriched pathways, and other functional modules as determined by RNA-Seq analysis, in human immune cells at Days 2, 4, and 29 after study vaccination comparing baseline changes in RNA expression between treatment arms (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

4. Protein abundance, clusters of co-abundant proteins, enriched pathways, and other functional modules as determined by quantitative proteomics analysis, in human immune cells at Days 2, 4, and 29 after first study vaccination comparing baseline changes (Days -7, and 1) in protein abundance between treatment arms (A/H3N2v vaccine vs. A/H7N9 vaccine and A/H3N2v vaccine vs. A/H7N9 vaccine + AS03).

5. Changes from baseline (Days -7, 1, and 29) in RNA expression or protein abundance that best correlate with influenza A/H7N9 seroconversion and with peak HAI titers at Days 2 and 4 (following first study vaccination) as well as Days 30, 32, and 36 (following second study vaccination).

6. Changes from baseline (Days -7, 1, and 29) in RNA expression or protein abundance that best correlate with baseline changes in cytokine concentrations at Days 2, 4, 8 (A/H3N2v arm only) and 29 (following first study vaccination) as well as Days 30, 32, and 36 (following second study vaccination).

7. Exploratory summaries of transcriptomics/proteomics response signatures for subjects with unexpected or severe safety profiles.

8. Lists of differential genes and proteins identified as part of the primary and secondary objectives.

4 STUDY DESIGN

This is a single center, randomized, partially-blinded, Phase II, small, targeted, prospective study in approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, designed to evaluate and compare the immunogenicity between an intramuscular monovalent inactivated influenza A/H7N9 virus vaccine manufactured by Sanofi Pasteur given with and without AS03 adjuvant manufactured by GlaxoSmithKline, and an intramuscular unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur.

This study will use a standard and systems biology approach to assess the human early gene and protein signatures expressed at baseline (approximately Days -7 and 1), and at approximately Days 2, 4, and 8 (A/H3N2v-vaccinated subjects only) after the first study vaccination in each treatment arm as well as at approximately Days 29, 30, 32, and 36 in A/H7N9-vaccinated subjects only. Cellular immunogenicity (systems biology studies) data will be integrated with serologic immunogenicity (HAI and Neut antibody assays) and reactogenicity data to develop a systems model of the human immune response to unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine and monovalent inactivated influenza A/H7N9 virus vaccine given with and without AS03 adjuvant.

This study will use venous blood samples and subject data collected from a total of thirty vaccinated subjects randomly divided into three equal treatment arms. The first treatment arm (n=10) will be vaccinated with one dose of 15 µg of A/H3N2v HA. The second treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given with AS03 approximately 28 days apart. The third treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given without AS03 adjuvant approximately 28 days apart. All study vaccinations will be administered intramuscularly.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study vaccination through 8 days after each study vaccination. Unsolicited non-serious adverse events (AEs) will be collected from the time of each study vaccination through approximately 28 days after each study vaccination. Serious adverse events (SAEs) and medically-attended adverse events (MAAEs) including new-onset chronic medical conditions (NOCMCs) and potentially immune-mediated medical conditions (PIMMCs) will be collected from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after the last study vaccination.

Venous blood samples (approximately 90 mL) will be collected from subjects at approximately Days -7 and 1 (immediately prior to the first study vaccination), and at approximately Days 2, 4;

and 8 (A/H3N2v-vaccinated subjects only) after the first study vaccination for systems biology studies (cytokine and chemokine levels, immune cell activation status, and whole transcriptome and proteome profiles of the major blood immune cells). Additionally, subjects in either treatment arm receiving A/H7N9 vaccine will have additional blood samples collected at approximately Days 29 (this will be collected immediately prior to the second study vaccination for subjects in either group receiving A/H7N9), 30, 32, and 36 after the first study vaccination for systems biology studies.

Serological assessment (hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays) will also be conducted on venous blood samples (approximately 10 mL) collected from subjects at Day 1 (immediately prior to the first study vaccination), and at approximately Days 29 (for A/H7N9-vaccinated subjects this will be collected immediately prior to the second study vaccination) and 57 (A/H7N9-vaccinated subjects only) after the first study vaccination.

We will quantify and characterize serum cytokine/chemokine levels, as well as transcriptomic and proteomic profiles from individual immune cell compartments, comparing this with standard serologic assessment to vaccine.

Urine samples (approximately 20 mL) will also be collected from subjects at approximately Days -7, 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29, 30, 32, and 36 for future research. *Note: Days 29, 30, 32, and 36 will apply only to A/H7N9-vaccinated subjects.*

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, who meet all eligibility criteria will be enrolled in this study from one participating VTEU site. Enrollment will occur over a 6-month period, and the target population will reflect the community at large. Information regarding this study may be mailed or emailed 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 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 Eligibility Criteria

5.1.1 Subject Inclusion Criteria

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

1. Provide written informed consent prior to initiation of any study procedures.
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, 18 to 49 years old, inclusive.
4. Are in good health[1].

[1]*As determined by medical history and targeted physical examination, if indicated based on medical history, to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, that 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. 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: Topical, nasal, and inhaled medications (with the exception of inhaled corticosteroids as outlined in the Subject Exclusion Criteria (see Section 5.1.2)), herbals, vitamins, and supplements are permitted.

5. Oral temperature is less than 100.4°F.
6. Pulse is 50 to 115 bpm, inclusive.
7. Systolic blood pressure is 85 to 150 mm Hg, inclusive.
8. Diastolic blood pressure is 55 to 95 mm Hg, inclusive.
9. Erythrocyte sedimentation rate (ESR) is less than 30 mm per hour.
10. Women of childbearing potential[2] must use an acceptable contraception method[3] from 30 days before first study vaccination until 60 days after last study vaccination.

[2]*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 or <1 year of the last menses if menopausal.*

[3]*Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the first study vaccination, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").*

11. Women of childbearing potential must have a negative urine or serum pregnancy test within 24 hours prior to study vaccination.

5.1.2 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 principal investigator or appropriate sub-investigator, within 72 hours prior to 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 principal investigator 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 principal investigator 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 (excluding non-melanoma skin cancer) or a history of any hematologic malignancy.

5. Have known human immunodeficiency virus (HIV), hepatitis B, or hepatitis C infection.

6. Have known hypersensitivity or allergy to eggs, egg or chicken protein, squalene-based adjuvants, or other components of the study vaccines.

7. Have a history of severe reactions following previous immunization with licensed or unlicensed influenza virus vaccines.

8. Have a personal or family history of narcolepsy.

9. Have a history of Guillain-Barré syndrome.

10. Have a history of convulsions or encephalomyelitis within 90 days prior to study vaccination.

11. Have a history of a potentially immune-mediated medical condition[6].

[6]*Refer to Appendix A: List of Potentially Immune-Mediated Medical Conditions.*

12. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.

13. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.

14. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.

15. Have taken oral or parenteral (including intra-articular) corticosteroids of any dose within 30 days prior to study vaccination.

16. Have taken high-dose inhaled corticosteroids within 30 days prior to study vaccination. High-dose defined as >840 mcg/day of beclomethasone dipropionate CFC or equivalent.

17. Received licensed live vaccine within 30 days prior to the first study vaccination, or plans to receive licensed live vaccine within 30 days before or after each study vaccination.

18. Received licensed inactivated vaccine within 14 days prior to the first study vaccination, or plans to receive licensed inactivated vaccine within 14 days before or after each study vaccination.

19. Received immunoglobulin or other blood products (with exception of Rho D immunoglobulin) within 90 days prior to study vaccination.

20. Received an experimental agent[7] within 30 days prior to the first study vaccination, or expects to receive an experimental agent[8] during the 13-month study-reporting period.

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

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

21. Are participating or plan to participate in another clinical trial with an interventional agent[9] that will be received during the 13-month study-reporting period.

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

22. Prior participation in a clinical trial of influenza A/H7 vaccine[10] or have a history of influenza A/H7 virus actual or potential exposure or infection prior to the first study vaccination.

    [10]*And assigned to a group receiving influenza A/H7 vaccine, does not apply to documented placebo recipients.*

23. Prior participation in a clinical trial of influenza A/H3N2v vaccine[11] or have a history of influenza A/H3N2v virus actual or potential exposure or infection prior to the first study vaccination.

    [11]*And assigned to a group receiving influenza A/H3N2v vaccine, does not apply to documented placebo recipients.*

24. Occupational exposure to or substantial direct physical contact[12] with birds in the past year or during the 28 days after each study vaccination.

    [12]*Casual contact with birds at petting zoos or county or state fairs or having pet birds does not exclude subjects from study participation.*

25. Occupational exposure to or substantial direct physical contact[13] with pigs in the past year or during the 28 days after each study vaccination.

    [13]*Casual contact with pigs at petting zoos or county or state fairs does not exclude subjects from study participation.*

26. Female subjects who are breastfeeding or plan to breastfeed at any given time from the first study vaccination until 30 days after the last study vaccination.

27. Plan to travel outside the US (continental US, Hawaii, and Alaska) within 28 days after each study vaccination.

28. Blood donation or planned blood donation within 30 days before enrollment until 30 days after the last blood draw for this study.

5.2 Treatment Assignment Procedures

5.2.1 Enrollment and Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the participating VTEU site to document the reason why an individual was screened, but failed study entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and 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 study, the subject will be enrolled. Subjects will be assigned randomly to 1 of 3 treatment arms. The first treatment arm (n=10) will be vaccinated with one dose of 15 µg of A/H3N2v HA. The second treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given with AS03 approximately 28 days apart. The third treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given without AS03 adjuvant approximately 28 days apart. All study vaccinations will be administered intramuscularly.

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC^{SM}. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for this study. 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 participating 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

participating VTEU site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subjects who sign the informed consent form and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form, and are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from this study, or are lost to follow-up may be replaced. The randomization scheme for this study is presented in the table below.

Treatment Arms

Treatment Arms (n=10 vaccinated subjects per Treatment Arm)	First Study Vaccination (Day 1)	Second Study Vaccination (Day 29±2 days)
1	15 µg A/H3N2v*	N/A
2	3.75 µg A/H7N9 + AS03	3.75 µg A/H7N9 + AS03
3	3.75 µg A/H7N9*	3.75 µg A/H7N9*
Total N=30 vaccinated subjects		

* unadjuvanted

5.2.2 Masking Procedures

This is a partially-blind clinical study as the unadjuvanted A/H3N2v vaccine treatment arm will be open-label to avoid the need for placebo administration at approximately Day 29. Investigators and study personnel will remain blinded to allocation of A/H7N9 with or without AS03.

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 member 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.

Laboratory personnel performing antibody, cytokine\chemokine, and RNA-Seq assays will be masked to A/H7N9 and A/H3N2v treatment groups, subject ID, and study visit for all study specimens. For proteomics assays, A/H7N9 treatment groups and study visit will be masked. Subject IDs will be replaced by random IDs generated by the SDCC to allow proteomics batch-processing of specimens obtained from the same subject. In all cases, the SDCC will generate random specimen picklists for each laboratory to determine which specimens will be tested. After all subjects have completed Day 57, the database will be locked to facilitate data analysis. At the discretion of DMID, unmasking of laboratories may occur after all data have been locked, analyzed and interpreted and the final CSR has been completed.

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.

5.2.3 Reasons for Withdrawals and Discontinuation of Treatment

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

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

- Medical disease or condition, or any new clinical findings 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 study, or would interfere with the evaluation of responses.
- Subject no longer meets eligibility criteria (see Section 5.1). Note: Medication changes within 60 days, as specified in Subject Inclusion Criterion #4, are exclusionary for receipt of the first study vaccination only.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of this study.
- New information becomes available that makes further participation unsafe.

The second study vaccination will not be administered to an A/H7N9-vaccinated subject if any of the following criteria are met:

- Medical condition or medication change for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity. For subjects with injection site or systemic signs or symptoms, or with an acute illness, including an oral temperature greater than or equal to 100.4°F, the second study vaccination should be postponed/deferred until signs, symptoms, or acute illness have resolved, or are improving as further specified below, and if within the acceptable protocol-specified window for that visit (Day 29±2 days post first study

vaccination). No exceptions to the protocol-specified window will be made. **Note for afebrile, acute illness only:** If a subject is afebrile, his/her acute illness is nearly resolved with only minor residual symptoms remaining, this occurs within the acceptable protocol-specified window for that visit (Day 29±2 days post first study vaccination), and, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol, the subject may receive the second study vaccination without further approval from the DMID Medical Officer. No exceptions to the protocol-specified window will be made.

- Grade 2 or 3 solicited or unsolicited adverse event that is ongoing, whether or not it is improved or resolving. An unresolved or continuing Grade 1 adverse event is permissible following the documented determination by the site principal investigator or appropriate sub-investigator, that it would not render study vaccination unsafe or interfere with the evaluation of responses.
- Grade 3 solicited or unsolicited adverse event that occurs without alternative etiology in the 8 days following the first study vaccination.
- Severe or sustained reaction or disability related to the first study vaccination.
- New onset of illness or condition that meets the Subject Exclusion Criteria (see Section 5.1.2).
- Subject no longer meets eligibility criteria (see Section 5.1). Note: Medication changes subsequent to the first study vaccination are not exclusionary for receipt of the second study vaccination 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.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject refusal of further study vaccination.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of this study.
- New information becomes available that makes further participation unsafe.

5.2.4 Handling of Withdrawals and Discontinuation of Treatment

The primary reason for withdrawal from this study 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 7.3.

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 5.2.3), those subjects who receive only one dose of study vaccine will be encouraged to remain in this study 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 12 months after their last study vaccination. These subjects will also be encouraged to provide a venous blood sample for serological assessment (HAI and Neut antibody assays) at approximately 28 days after their last study vaccination. Collection of venous blood samples for systems biology studies will be obtained at serial time points indicated per the study schedule, if possible. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Every attempt will be made to follow all adverse events, including solicited injection site and systemic reactions, unsolicited non-serious adverse events, serious adverse events, and medically-attended adverse events including new-onset chronic medical conditions and potentially immune-medicated medical conditions, ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of adverse event.

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, e-mails, 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.

Subjects who withdraw, or are withdrawn or terminated from this study, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine may be replaced. Similarly, subjects in the A/H7N9 study arms who do not receive their second vaccination will be replaced to ensure that at least 10 evaluable subjects per A/H7N9 study arm are included in the analyses.

5.2.5 Termination of Study

Although the sponsor has every intention of completing this study, it reserves the right to terminate this study 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

A/H7N9 Vaccine

Sanofi Pasteur has developed a monovalent inactivated influenza A/H7N9 virus vaccine for intramuscular use. The manufacturing process for the production of this monovalent inactivated influenza A/H7N9 virus vaccine is similar to the manufacturing process for the production of its licensed Influenza Virus Vaccine Fluzone® family of products, except for a minor modification in the PBS diluent in the formulation step that was made according to previous experiences of manufacturing of monovalent pandemic vaccines. This monovalent inactivated split influenza virus vaccine was derived from the influenza A/Shanghai/2/2013 virus. Licensed release testing specifications were maintained, with a modification to the potency test. [redacted] We plan to use a 3.75 mcg of HA (A/Shanghai/2/2013) per 0.5 mL [adjuvanted] or 0.25 mL [unadjuvanted] dose in this study.

AS03 Adjuvant [Adjuvant System (03)]

[redacted]

A/H3N2v Vaccine

Sanofi Pasteur has developed a monovalent inactivated influenza A/H3N2v. The resulting reassortant is designated A/Minnesota/11/2010 NYMC X-203 virus vaccine for intramuscular use. The manufacturing process for the production of this monovalent

inactivated influenza A/H3N2v virus vaccine is similar, with slight modifications in the formulation step, to the manufacturing process for the production of its licensed Influenza Virus Vaccine Fluzone® and the H1N1 influenza vaccine A/California/07/2009 NYMC X-179A. Using the Fluzone® production process, an A/H3N2v reassortant was derived from the A/Minnesota/11/2010 strain produced by classical reassortant technology at New York Medical College (NYMC) and provided by the Centers for Disease Control and Prevention (CDC). This monovalent inactivated split influenza virus vaccine is prepared from influenza virus propagated in embryonated chicken eggs. Licensed release testing specifications were maintained.

6.1.1 Acquisition

A/H7N9 vaccine will be provided by Sanofi Pasteur under contract to BARDA/DHHS.

A/H3N2v vaccine will be provided by Sanofi Pasteur under contract to BARDA/DHHS.

AS03 adjuvant will be provided by GlaxoSmithKline Biologicals under contract to BARDA/DHHS.

Upon request by DMID, A/H7N9 vaccine, AS03 adjuvant, and A/H3N2v vaccine will be transferred to the following address:

DMID Clinical Agents Repository Contract
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
[redacted]

Sterile empty vials will be obtained by the DMID Clinical Agents Repository (CAR), Fisher BioServices.

A/H7N9 vaccine, AS03 adjuvant, A/H3N2v vaccine, and sterile empty vials for study vaccine preparation will be provided through the DMID CAR to the participating VTEU site prior to the start of this study upon request and with prior approval from DMID. Should the site principal investigator require additional A/H7N9 vaccine, AS03 adjuvant, A/H3N2v vaccine, or sterile empty vials during this study, further instructions are provided in the protocol-specific MOP.

6.1.2 Formulation, Storage, Packaging, and Labeling

A/H7N9 Vaccine

The monovalent inactivated influenza A/H7N9 virus vaccine is supplied as a sterile, clear, and slightly opalescent suspension in single-dose vials containing 7.5 mcg of HA per 0.5 mL. Each vial contains a fill volume of 0.7 mL. It contains no preservative (i.e., non-thimerosal) or antibiotics. The vials containing study product must be stored at 2°C to 8°C (35.6°F to 46.4°F). Do not freeze. Vials will be provided with latex-free stoppers.

AS03 Adjuvant

The AS03 adjuvant is supplied as a preservative-free, oil-in-water, whitish to yellowish homogenous milky liquid emulsion in single-use vials containing a fill volume of 3.15 mL. The vials containing study product must be stored at 2°C to 8°C (35.6°F to 46.4°F). Do not freeze. Vials will be provided with latex-free stoppers.

A/H3N2v Vaccine

The monovalent inactivated influenza A/H3N2v virus vaccine is supplied as a sterile, clear, and slightly opalescent suspension. A minor modification in the PBS diluent in the formulation step was made to accommodate the nature of the diluted virus in single-dose, prefilled syringes, without needles, containing 15 mcg of HA per 0.5mL. It contains no preservative (i.e., non-thimerosal), antibiotics, or latex. The vaccine includes porcine gelatin (0.05%) as a stabilizer. The prefilled syringes containing study product must be stored at 2°C to 8°C (35°F to 46°F). Do not freeze.

Each of these study products will be labeled according to manufacturer specifications and include the statement “Caution: New Drug – Limited by Federal Law to Investigational Use.”

Further details are included in the respective, applicable Investigator’s Brochures and Supplemental Information for the Investigator’s Brochures for the A/H7N9 vaccine, AS03 adjuvant, and A/H3N2v vaccine as well as protocol-specific MOP.

Sterile empty vials will be provided with latex-free stoppers.

6.1.3 Study Product Storage and Stability Procedures

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 study 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 CAR 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 B and C for detailed information on the preparation, labeling, storage, and administration of A/H7N9 vaccine given with or without AS03 adjuvant. Study vaccine preparation will be performed by the participating VTEU site's research pharmacist on the same day of study vaccine administration. No study vaccine preparation is required for the A/H3N2v vaccine as it is contained in a prefilled syringe and the entire contents will be administered to subjects per treatment assignment.

Visually inspect the A/H7N9 vaccine, AS03 adjuvant, and A/H3N2v vaccine upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at 2°C to 8°C (35.6°F to 46.4°F) 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 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 CAR or destroy it on site. If the A/H7N9 vaccine, AS03 adjuvant, or A/H3N2v vaccine is unusable, study personnel will use another vial or prefilled syringe from the study supply. Replacement vials or prefilled syringes may be requested by

contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Also, visually inspect the A/H7N9 vaccine plus AS03 adjuvant admixture prior to use. The admixture will be milky (whitish to yellowish) in appearance. If it appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use it. The affected A/H7N9 vaccine plus AS03 adjuvant admixture must be quarantined at room temperature 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 A/H7N9 vaccine plus AS03 adjuvant admixture can be used. If it cannot be used, the site will receive specific instructions on how to send the A/H7N9 vaccine plus AS03 adjuvant admixture to the DMID CAR or destroy it on site. If the A/H7N9 vaccine plus AS03 adjuvant admixture is unusable, the participating VTEU site's research pharmacist will prepare another vial. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Once mixed, the A/H7N9 vaccine plus AS03 adjuvant admixture must be stored at room temperature in an upright position and must be used within 8 hours.

Study vaccine administration will be performed by an unblinded study team member 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. Each dose of study vaccine will be administered to subjects via a single IM injection into the deltoid muscle of the preferred arm on the day of each study vaccination. Study vaccinations subsequent to the first study vaccination may be given in the same preferred arm as long as there is no interference with the reactogenicity assessment. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Aseptic technique will be used for the withdrawal and administration of each dose of study vaccine using a disposable, sterile needle appropriate in length for each subject and a 1-mL disposable, sterile syringe (except for the A/H3N2v vaccine as it is contained in a prefilled syringe). See the protocol-specific MOP for information on how to administer IM injections. For the A/H7N9 vaccine and A/H7N9 vaccine plus AS03 adjuvant admixture, each dose of study vaccine must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must remain at room temperature until administered. Note: Each 0.5 mL dose of AS03-adjuvanted A/H7N9 vaccine contains one- 0.25 mL dose of AS03 adjuvant.

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 29 ± 2 days post first study vaccination). No exceptions to the protocol-specified window will be made.

Subjects who do not receive the second study vaccination will continue with follow-up safety assessments (may be conducted by phone call/electronic communication (e.g., email, text message) rather than in person) continuing through approximately 12 months after their first study vaccination. These subjects will also be encouraged to provide a venous blood sample for serological assessment (HAI and Neut antibody assays) at approximately 28 days after their first study vaccination. See Sections 5.2.3 and 5.2.4 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 A/H7N9 vaccine, AS03 adjuvant, A/H3N2v vaccine, and sterile empty vials, 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, as appropriate for type of study product, vial numbers, including final mixed vial number, date of study vaccine preparation/administration, time of study vaccine preparation, expiration of study vaccine preparation, time study vaccine is drawn into the syringe, expiration time of study vaccine 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 A/H7N9 vaccine, AS03 adjuvant, A/H3N2v vaccine, and A/H7N9 vaccine plus AS03 adjuvant admixture, 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 A/H7N9 vaccine, AS03 adjuvant, and A/H7N9 vaccine plus AS03 adjuvant admixture and unused syringes of A/H3N2v vaccine will be retained until monitored and released for disposition as applicable. This can occur on an ongoing basis for used vials of A/H7N9 vaccine, AS03 adjuvant, and A/H7N9 vaccine plus AS03 adjuvant admixture. Used syringes of A/H3N2v vaccine will be discarded immediately after administration. Final disposition of the unused A/H7N9 vaccine, AS03 adjuvant, A/H3N2v vaccine, and sterile empty vials will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

Study product will be administered to subjects by an unblinded study vaccine administrator via IM injection at all dosing times according to subject treatment assignment and as described in Section 6.2. Thus, subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in Section 6.3.

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 within 60 days prior to signing the informed consent form through approximately 28 days after the last study vaccination or through the early termination visit (if prior to 28 days after the last 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 28 days after the last 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 28 days after the last study vaccination, and reported in the eCRF.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of the investigational product(s) should not be used during the study-reporting period (approximately 12 months after the last study vaccination) unless clinically indicated as part of the subject's health care. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 5.1.2). 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.

7 STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4 and 8 and Appendix B.

7.1 Screening and Enrollment Visits

7.1.1 Visit 00, Day -7, Screening, Clinic Visit, All Subjects (Window: Day -14 to Day -5 prior to first study vaccination)

Potential subjects will be screened for eligibility up to 14 days, but no less than 5 days prior to the administration of the first study vaccination. The following activities will be performed:

- Subjects will be provided with a description of this study (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, including any screening procedures.
- Demographic information will be obtained by interview of subjects.
- Eligibility criteria will be reviewed with subjects.
- Complete medical history will be obtained by interview of subjects to ensure eligibility.
- All concomitant medications taken within 60 days prior to signing the informed consent form will be reviewed with subjects. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination.
- Subject receipt of licensed seasonal influenza vaccines 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 5.1.2).
- Vital signs, including oral temperature, pulse, and blood pressure, 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 targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, may be performed, if indicated based on

review of complete 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 urine or serum pregnancy test will be performed on all women of childbearing potential and must be negative to ensure eligibility.

- Approximately 4 mL of venous blood will be collected for ESR, and performed locally by the site. The ESR value must be confirmed as less than 30 mm per hour prior to randomization and first study vaccination. The ESR, if initially elevated, may be repeated once to determine eligibility.

- Approximately 90 mL of venous blood will be collected for baseline cytokine and chemokine levels and systems biology studies.

- Approximately 20 mL of urine will be collected for future research.

7.1.2 Visit 01, Day 1, Enrollment and First Study Vaccination (Dose 1), Clinic Visit, All Subjects

- Subject's willingness to participate will be reconfirmed and documented in the subject's study records prior to performing any further study procedures, including administration of the first study vaccination.

- Eligibility criteria, including results of the ESR evaluation, will be reviewed with subjects prior to the first study vaccination to ensure continued eligibility.

- Complete medical history and any updates obtained by interview of subjects since the screening visit will be reviewed with subjects prior to the first study vaccination to ensure continued eligibility.

- All concomitant medications will be reviewed with subjects prior to the first study vaccination for accuracy and completeness. Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility prior to the first study vaccination. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the first study vaccination. Vital signs assessed on Day 1 prior to the first study

vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- Height and weight will be collected prior to the first study vaccination for the calculation of Body Mass Index (BMI).

- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, may be performed prior to the first study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of subjects since the screening visit, 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 urine or serum pregnancy test will be performed within 24 hours prior to the first study vaccination on all women of childbearing potential. Results must be negative and known prior to randomization and first study vaccination.

- Approximately 10 mL of venous blood will be collected immediately prior to the first study vaccination for baseline HAI and Neut antibody assays.

- Approximately 90 mL of venous blood will be collected immediately prior to the first study vaccination for baseline cytokine and chemokine levels and systems biology studies.

- Approximately 20 mL of urine will be collected prior to the first study vaccination for future research.

- 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 vaccine via IM injection into the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the first study vaccination. The first study vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.

- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their 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. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects 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 subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.2 Follow-up Visits

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

7.2.1 Visit 02, Day 2, Clinic Visit, All Subjects (Window: Day 2+1 day post first study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.
- Memory aid information will be reviewed with subjects.
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form.
- All AE/SAEs will be recorded on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.
- The first study vaccination site will be examined.

- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.

- Approximately 20 mL of urine will be collected for future research.

7.2.2 Visit 03, Day 4, Clinic Visit, All Subjects (Window: Day 4+1 day post first study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects.

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

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

- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.

- The first study vaccination site will be examined.

- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.

- Approximately 20 mL of urine will be collected for future research.

7.2.3 Visit 04, Day 8, Clinic Visit, A/H3N2v-vaccinated subjects only (Window: Day 8+2 days post first study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects.
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form.
- All AE/SAEs will be recorded on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.
- The first study vaccination site will be examined.
- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.
- Approximately 20 mL of urine will be collected for future research.

7.2.4 Visit 04, Day 8, Phone Call/Electronic Communication, A/H7N9-vaccinated subjects only (Window: Day 8+2 days post first study vaccination)

Study personnel will contact subjects by phone or electronic communication (e.g., email, text message) to solicit any AE/SAE and concomitant medication information (including solicitation for receipt of any non-study influenza vaccines) and review information on their memory aid.

7.2.5 Visit 05, Day 29, *Second Study Vaccination (Dose 2) for A/H7N9-vaccinated subjects only, Clinic Visit, All Subjects (Window: Day 29±2 days post first study vaccination)

***This visit is for all subjects; however only A/H7N9-vaccinated subjects will receive the second study vaccination (Dose 2).**

- A/H7N9-vaccinated subjects only – Eligibility criteria, will be reviewed with subjects prior to the second study vaccination* to ensure continued eligibility.
- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be

obtained by interview of subjects (prior to the second study vaccination*) and note 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*).

- All AE/SAEs will be recorded on the appropriate data collection form (prior to the second study vaccination*).

- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the second study vaccination*. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Note: Vital signs are not required for A/H3N2v-vaccinated subjects or A/H7N9-vaccinated subjects who are discontinued from receipt of the second study vaccination and are being followed for safety.

- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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/H7N9-vaccinated subjects only – A urine or serum pregnancy test will be performed within 24 hours prior to the second study vaccination* on all women of childbearing potential. Results must be negative and known prior the second study vaccination*.

- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays on all subjects. A/H7N9-vaccinated subjects only – Blood should be collected immediately prior to the second study vaccination*.

- A/H7N9-vaccinated subjects only – Approximately 90 mL of venous blood will be collected immediately prior to the second study vaccination* for cytokine and chemokine levels and systems biology studies.

- A/H7N9-vaccinated subjects only - Approximately 20 mL of urine will be collected (prior to the second study vaccination*) for future research.

- A/H7N9-vaccinated subjects only – Pre-administration reactogenicity assessments will be performed prior to the second study vaccination* to establish baseline. Subjects will then

receive a single dose of study vaccine via IM injection into the deltoid muscle of the preferred arm. The second study vaccination may be given in the same preferred arm as long as there is no interference with the reactogenicity assessment. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the second study vaccination. The second study vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.

- A/H7N9-vaccinated subjects only – Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their 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. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after the second study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

Note: This will be the last visit for those randomized to the A/H3N2v arm.

7.2.6 Visit 06, Day 30, Clinic Visit, A/H7N9-vaccinated subjects only (Window: Day 2+1 day post second study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects.

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

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

- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.

- The second study vaccination site will be examined.
- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.
- Approximately 20 mL of urine will be collected for future research.

7.2.7 Visit 07, Day 32, Clinic Visit, A/H7N9-vaccinated subjects only (**Window**: **Day 4+1 day post second study vaccination)**

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.
- Memory aid information will be reviewed with subjects.
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form.
- All AE/SAEs will be recorded on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.
- The second study vaccination site will be examined.
- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.
- Approximately 20 mL of urine will be collected for future research.

7.2.8 Visit 08, Day 36, Clinic Visit, A/H7N9-vaccinated subjects only (Window: Day 8+2 days post second study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.
- Memory aid information will be reviewed with subjects.
- All concomitant medications (including solicitation for receipt of any non-study influenza vaccines) will be recorded on the appropriate data collection form.
- All AE/SAEs will be recorded on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.
- The second study vaccination site will be examined.
- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies.
- Approximately 20 mL of urine will be collected for future research.

7.2.9 Visit 09, Day 57, Clinic Visit, A/H7N9-vaccinated subjects only (Window: Day 29±2 days post second study vaccination)

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects 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.
- All AE/SAEs will be recorded on the appropriate data collection form.
- A targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.
- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays.

7.2.10 Visit 10, Day 209, Phone Call/Electronic Communication, A/H7N9-vaccinated subjects only (Window: Day 181±14 days post second study vaccination)

Subjects will be contacted by phone or electronic communication (e.g., email, text message) to query for safety events. Adverse events limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions that have occurred since the previous clinic visit or contact will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

7.2.11 Visit 11, Day 394, Phone Call/Electronic Communication, A/H7N9-vaccinated subjects only (Window: Day 366±14 days post second study vaccination)

Subjects will be contacted by phone or electronic communication (e.g., email, text message) to query for safety events. Adverse events limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions that have

occurred since the previous clinic visit or contact will be solicited. Based on the information, subjects may be asked to return to the clinic for evaluation.

7.3 Early Termination Visit (if needed), All Subjects

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

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects (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 prior to 28 days after the last study vaccination).

- All AE/SAEs will be recorded on the appropriate data collection form. AEs will be limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions if after 28 days following the last study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, 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 targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.

- The study vaccination site will be examined (if within 8 days after the last study vaccination).

- Post-administration reactogenicity assessments will be performed (if within 8 days after the last study vaccination).

- Approximately 10 mL of venous blood will be collected for HAI and Neut antibody assays (if prior to 28 days after the last study vaccination).

- Approximately 90 mL of venous blood will be collected for cytokine and chemokine levels and systems biology studies (if prior to 28 days after the last study vaccination).

- Approximately 20 mL of urine will be collected for future research (if prior to 28 days after the last study vaccination).

7.4 Unscheduled Visit (if needed), All Subjects

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

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted (if indicated).

- Memory aid information will be reviewed with subjects (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 prior to 28 days after the last study vaccination).

- All AE/SAEs will be recorded on the appropriate data collection form. AEs will be limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions if after 28 days following the last study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, 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 targeted physical examination, including an assessment for signs suggestive of a potentially immune-mediated medical condition, 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.

- The study vaccination site will be examined (if within 8 days after the last study vaccination).

- Post-administration reactogenicity assessments will be performed (if within 8 days after the last study vaccination).

8 STUDY PROCEDURES AND EVALUATIONS

8.1 Clinical Evaluations

Complete medical history will be obtained by interview of subjects at the screening visit and will be updated on Day 1 prior to the first study vaccination. Subjects 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 noting any changes since the previous clinic visit or contact. The interim medical history should include an assessment for new medical conditions and symptoms suggestive of a potentially immune-mediated medical condition.

Medications history (concomitant medications) will include a review of all current medications and medications taken within 60 days prior to signing the informed consent form through approximately 28 days after the last study vaccination or through the early termination visit (if prior to 28 days after the last study vaccination), whichever occurs first. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination through approximately 28 days after the last 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 28 days after the last study vaccination, and reported in the eCRF. Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Sections 5.1.1 and 5.1.2). 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 targeted physical examination may be performed, if indicated based on subject's complete 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. On Day 1 prior to the first study vaccination and at follow-up visits after the first study vaccination, a targeted physical examination may be performed, if indicated based on 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. Targeted physical examinations

should also include an assessment for signs suggestive of a potentially immune-mediated medical condition.

Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit and prior to each study vaccination. Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Height and weight will be collected on Day 1 prior to the first study vaccination for the calculation of BMI.

Reactogenicity assessments will include an assessment of solicited adverse events occurring from the time of each study vaccination through 8 days after each study vaccination, which includes an assessment of injection site reactions including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness as well as systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, and nausea. Pre-administration reactogenicity assessments will be performed prior to each study vaccination to establish baseline, then the study vaccination will be given.

Subjects will be observed in the clinic for at least 20 minutes after each study vaccination. The study vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic. The study vaccination site will also be examined at follow-up visits occurring through approximately 8 days after each study vaccination.

All subjects will complete a subject memory aid from the time of each study vaccination through 8 days after each study vaccination. Subject memory aids will be reviewed with the subjects for adverse events (solicited injection site and systemic reactions and unsolicited AEs) at follow-up visits occurring through approximately 8 days after each study vaccination.

8.2 Laboratory Evaluations

The volume of venous blood to be collected for ESR, HAI and Neut antibody assays, cytokine and chemokine levels, and systems biology studies is presented in the table below.

Venipuncture Volumes (mL)

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04^**	**V05***	**V06~**	**V07~**	**V08~**	**V09~**
Study Day post first study vaccination	**Screening D-7d (Day -14 to Day -5)**	**Enrollment and Dose 1 D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	***D30***	***D32***	***D36***	***D57***
Study Day post second study vaccination						**Dose 2 D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**
Study Vaccination		X				X~				
ESR	4									
HAI and Neut Antibody Assays		10†				10†				10
Cytokine and Chemokine Levels	10	10†	10	10	10	10†~	10	10	10	
Systems Biology Studies	80	80†	80	80	80	80†~	80	80	80	
Total A/H3N2v arm	**94**	**100**	**90**	**90**	**90**	**10**	**0**	**0**	**0**	**0**
Total A/H7N9 arms	**94**	**100**	**90**	**90**	**0**	**100**	**90**	**90**	**90**	**10**

^ A/H3N2v-vaccinated subjects only.

* This visit is for all subjects; however only A/H7N9-vaccinated subjects will receive the second study vaccination (Dose 2).

~ A/H7N9-vaccinated subjects only.

† All blood drawn immediately prior to study vaccination.

8.2.1 Clinical Laboratory Evaluations

Urine or serum pregnancy tests will be performed locally by the site at the screening visit and within 24 hours prior to each study vaccination on all women 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 dose of study vaccine.

Clinical screening laboratory parameters to be evaluated to confirm study eligibility and receipt of the first study vaccination will include ESR. To be eligible for participation in this study and receipt of the first study vaccination, the subject's ESR evaluation must be confirmed to meet the eligibility criteria as outlined in the Subject Inclusion Criteria (see Section 5.1.1). The ESR

evaluation will be performed locally by the site. A venous blood sample (approximately 4 mL) will be collected from each subject at the screening visit.

8.2.2 Special Assays or Procedures

8.2.2.1 HAI and Neut Antibody Assays – Serologic Immunogenicity

Assays to determine serum levels of HAI and Neut antibodies will be performed at Southern Research. Venous blood samples (approximately 10 mL) for HAI and Neut antibody assays will be collected from subjects at Day 1 (immediately prior to the first study vaccination), and at approximately Days 29 (for A/H7N9-vaccinated subjects this will be collected immediately prior to the second study vaccination) and 57 (A/H7N9-vaccinated subjects only) after the first study vaccination. Subjects who withdraw early will have HAI and Neut antibody assays run on available sera, including sera from the early termination visit, if available.

The Vanderbilt VTEU site will remain blinded to the HAI and Neut antibody assay results performed at Southern Research until after all subjects have completed Day 57 visits and all serologic laboratory results are complete.

8.2.2.2 Systems Biology Studies – Cellular Immunogenicity

Venous blood samples collected from subjects at time points and amounts described below will be used to measure changes in cytokine and chemokine levels, immune cell activation status, and whole transcriptome and proteome profiles of major blood immune cells to identify and quantify changes in gene expression. We will use mathematical modeling of the accumulated data to identify ribonucleic acid (RNA) or protein expression signatures that correlate significantly with the observed serum HAI and Neut antibody response, cytokine and chemokine response, and immune cell activation response. Urine samples (approximately 20 mL) will also be collected from subjects at approximately Days -7 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29, 30, 32, and 36 for future research. *Note: Days 29, 30, 32, and 36 will apply only to A/H7N9-vaccinated subjects*. By comparing subjects vaccinated with A/H7N9 vaccine given with and without AS03 adjuvant, our study may identify biomarkers that indicate that the AS03 adjuvant has successfully enhanced the immunological response. We may also identify differences between receipt of A/H7N9 vaccine given with and without AS03 adjuvant and unadjuvanted A/H3N2v vaccine.

For systems biology analysis of the subject's immune and blood cells responses to unadjuvanted A/H3N2v vaccine and A/H7N9 vaccine given with and without AS03 adjuvant, cytokine and chemokine levels, immune cell activation status, and whole

transcriptome and proteome profiles of major blood immune cells will be determined in venous blood samples collected at approximately Days -7, 1, 2, 4, and 8 (A/H3N2v-vaccinated subjects only). These will also be performed on venous blood samples collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only. RNA transcript abundance in blood immune cells collected at approximately Days -7, 1, 2, 4, and 8 (A/H3N2v-vaccinated subjects only) will be determined by RNA-Seq and gene expression quantifications will be obtained by counting mapped reads. RNA-Seq will also be performed on blood immune cells collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only. Relative cellular protein changes in subject's blood immune cells collected at approximately Days -7, 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only) will be identified by shotgun proteomics and quantified using either Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) or Tandem Mass Tags (TMT)[53]. These will also be performed on blood immune cells collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only. Similar comparisons will also be made between those subjects receiving A/H7N9 vaccine given with and without AS03 adjuvant and those subjects receiving unadjuvanted A/H3N2v vaccine.

(a) Venous blood samples will be collected prior to the first study vaccination at approximately Days -7 and 1. The pre-study vaccination time points permit setting the baseline immune activity for each subject enrolled in this study. This is a significant advantage, because it will correct for baseline variations between subjects that are caused by uncontrollable parameters such as genomic and/or metagenomic variations, asymptomatic infections, personal habits (e.g., tobacco use), or even over-the-counter medications.

(b) Additional venous blood samples will be collected after the first study vaccination at approximately Days 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29 and 57 (A/H7N9-vaccinated subjects only) to monitor the serologic and cellular response to study vaccination. On Day 29, for A/H3N2v-vaccinated subjects, only serologic assays will be performed.

(c) For the approximately Days -7, 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), and 29 (A/H7N9-vaccinated subjects only) approximately 90 to 100 mL of venous blood will be collected for serological assessment (HAI and Neut antibody assays, as applicable), cytokine and chemokine levels, and systems biology studies. On Day 29, for A/H3N2v-vaccinated subjects, only serologic assays will be performed. For the final serological assessment at approximately Day 57, approximately 10 mL of venous blood will be collected from A/H7N9-vaccinated subjects only. The venous blood samples will be immediately processed to avoid possible sample variation

caused by freezing and thawing cells.

Urine samples (approximately 20 mL) will also be collected from subjects at approximately Days -7, 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29, 30, 32, and 36 for future research. *Note: Days 29, 30, 32, and 36 will apply only to A/H7N9-vaccinated subjects.*

Day -7: Baseline pre-study vaccination time point #1 – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 1: Baseline pre-study vaccination time point #2 (immediately prior to the first study vaccination) – approximately 10 mL for cytokine and chemokine levels + approximately 10 mL for HAI and Neut antibody assays + approximately 80 mL for systems biology studies = 100 mL of venous blood

Day 2: To monitor early innate immune response – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 4: To monitor intermediate innate immune response – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 8: A/H3N2v-vaccinated subjects only. To monitor late innate immune response & early adaptive immune response – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 29: A/H3N2v-vaccinated subjects – approximately 10 mL for HAI and Neut antibody assays

A/H7N9-vaccinated subjects. To monitor the mature adaptive immune response (this will be collected immediately prior to the second study vaccination) – approximately 10 mL for cytokine and chemokine levels + approximately 10 mL for HAI and Neut antibody assays + 80 mL for systems biology studies = 100 mL of venous blood

Day 30: A/H7N9-vaccinated subjects only. To monitor early innate immune response to second study vaccination – approximately 10 mL for

cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 32: A/H7N9-vaccinated subjects only. To monitor late-intermediate innate immune response to second study vaccination – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 36: A/H7N9-vaccinated subjects only. To monitor late innate immune response & early adaptive immune response to second study vaccination – approximately 10 mL for cytokine and chemokine levels + approximately 80 mL for systems biology studies = 90 mL of venous blood

Day 57: A/H7N9-vaccinated subjects only. Final serological assessment (HAI and Neut antibody assays) – approximately 10 mL for HAI and Neut antibody assays

(d) A fraction of the collected venous blood samples will be analyzed as follows in e-k to measure the immunological response signature for each subject in response to study vaccination. The emerging immunologic signatures will be used in systems biology modeling.

(e) **HAI- and Neut-specific Antibody Response:** HAI- and Neut-specific antibody response will be measured at specified time points (see above for HAI and Neut antibody assays) to confirm the effective influenza immunity and to correlate with the systems biology studies data. HAI and Neut antibody assays will be performed by Southern Research.

(f) **Cytokine and Chemokine Response:** Using the Vanderbilt Immunology Core, cytokine and chemokine levels in venous blood samples will be quantitatively measured at approximately Days -7, 1, 2, 4, and 8 (A/H3N2v-vaccinated subjects only), as listed above in (c). This will also be performed on venous blood samples collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only.

The majority of the collected venous blood samples will be used to isolate immune cell subsets to profile the transcriptome and proteome profiles in response to study vaccination. The gene expression signatures along with the immunologic signatures will be used in the systems biology approach to identify biomarkers or gene

expression signatures that predict an immunological response.

(g) **Fractionation of Peripheral Blood into Immune Cell Subsets:** Magnetic cell separation (MACS) using antibody-coated magnetic beads is a fast and efficient way to isolate specific immune cell populations directly from venous blood samples. Using Ficoll separation and immune subset marker-specific magnetic beads on the fresh venous blood samples, we will isolate 6 major immune cell subsets:

1. Neutrophils (Polymorphonuclear Leukocyte [PMN])
2. Monocytes
3. Natural Kill (NK) cells
4. B-cells
5. T-cells
6. Dendritic cells (DC)

All isolated immune cell subsets will be stored at -80°C. The subjects' samples will be used for in-depth systems biology analysis to optimize the discovery of gene expression signatures that predicts an early immunological response and protection. This will be performed by the Vanderbilt Immunology Core.

(h) **RNA-Seq Transcriptome Sequencing:** Transcriptome profiling using RNA-Seq will be performed on immune cell subsets collected at approximately Days -7, 1, 2, 4, and 8 (A/H3N2v-vaccinated subjects only), as listed above in (c) and (g) to identify and quantify the RNA transcript response after study vaccination. This will also be performed on immune cell subsets collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only. Total RNA will be isolated using standard methods and subjected to ultra-high-throughput parallel next generation sequencing. This will be performed by the Vanderbilt Immunology Core and Hudson Alpha.

(i) **Proteomics Profiling:** To identify and quantify changes in the immune cell's proteome after study vaccination, quantitative and shotgun proteomics using multidimensional liquid chromatography coupled with tandem mass spectrometry will be performed on immune cell samples collected at approximately Days -7, 1, 2, 4, and 8 (A/H3N2v-vaccinated subjects only), as listed above in (c) and (g). These will also be performed on immune cell subsets collected at approximately Days 29, 30, 32, and 36 from A/H7N9-vaccinated subjects only. This will be performed by the Vanderbilt Proteomics Core.

(j) **Systems Biology Analysis:** Our goal is to identify the unique RNA and protein biomarkers that correlate with the immunological responses from A/H7N9 vaccine given with and without AS03 adjuvant. We may also identify differences between

receipt of A/H7N9 vaccine given with and without AS03 adjuvant and unadjuvanted A/H3N2v vaccine.

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 protocol-specific MOP.

8.2.3.2 Specimen Shipment

Specimen shipment will occur at intervals during the course of this study 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 CAR, and then provided by the DMID CAR to Southern Research in a blinded manner once they all become available to the DMID CAR.

Specimens for systems biology studies will remain at the Vanderbilt VTEU site as experiments are being conducted. Once RNA is extracted from subjects' immune cell subsets, these RNA samples will be sent directly to HudsonAlpha for RNA-sequencing given the susceptible for sample degradation during multiple shipments. Any specimens remaining once the protocol-defined systems biology studies are completed will be shipped to the DMID CAR.

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

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Study vaccine-related serious adverse events occurring from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after the last study vaccination.

2. Solicited Adverse Events – reactogenicity events occurring from the time of each study vaccination through 8 days after each study vaccination:

   a) Injection site reactions including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness.

   b) Systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, and nausea.

3. Unsolicited Adverse Events – study vaccine-related non-serious adverse events occurring from the time of each study vaccination through approximately 28 days after each study vaccination.

4. Medically-Attended Adverse Events including new-onset chronic medical conditions and potentially immune-mediated medical conditions occurring from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after the last study vaccination.

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

9.2.1 Adverse Events

Adverse Event (AE): International Conference on Harmonisation (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 (injection site) 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 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). Adverse events 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.

Medically-Attended Adverse Events (MAAEs): For each unsolicited AE experienced, the subject will be asked if he/she had received medical attention, defined as hospitalization, an emergency room visit, or an otherwise unscheduled visit to or from medical personnel for any reason. AEs characterized by such unscheduled medical care will be designated as medically-attended adverse events. MAAEs can also include new-onset chronic medical conditions, or potentially immune-mediated medical conditions (also known as Adverse Events of Special Interest [AESIs]).

New-Onset Chronic Medical Conditions (NOCMCs): New-onset chronic medical conditions 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.

Potentially Immune-Mediated Medical Conditions (PIMMCs): Potentially immune-mediated medical conditions constitute a group of AEs that includes diseases which are clearly

autoimmune in etiology and other inflammatory and/or neurologic disorders which may or may not have autoimmune etiologies. PIMMCs currently in effect are presented in Appendix A: List of Potentially Immune-Mediated Medical Conditions.

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 study. 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 adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- 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 these types of study vaccines. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Local (Injection Site) Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain, but it does not interfere with daily activity, **and** no pain medication is taken	There is interference with daily activity **or** it requires repeated use of a non-narcotic pain reliever for >24 hours	Pain prevents daily activity or requires any use of a narcotic pain reliever
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
Pruritus (Itching)	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Ecchymosis (Bruising)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Erythema (Redness)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration (Hardness)/Swelling*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

* Will also be measured in mm but size will not be used as halting criteria.

Ecchymosis (bruising), erythema (redness), and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Small	Medium	Large
Ecchymosis (Bruising)*	<20 mm	20 mm – 50 mm	>50 mm
Erythema (Redness)*	<20 mm	20 mm – 50 mm	>50 mm
Induration (Hardness)/Swelling*	<20 mm	20 mm – 50 mm	>50 mm

* Will not be used as halting criteria.

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
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site.

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 Additional Adverse Event Severity Grading

Pulse and blood pressure[#] will be graded as follows:

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	45 – 49	40 – 44	<40
Tachycardia - beats per minute	116 – 130	131 – 155	>155
Hypotension (systolic) mm Hg	80 – 84	75 – 79	<75
Hypotension (diastolic) mm Hg	50 – 54	45 – 49	<45
Hypertension (systolic) mm Hg	151 – 155	156 – 160	>160
Hypertension (diastolic) mm Hg	96 – 100	101 – 105	>105

[#] Pulse and blood pressure assessed on Day 1 prior to the first study vaccination will be considered as baseline.

9.2.4 Serious Adverse Events

Serious Adverse Event (SAE): An adverse event 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 adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require 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.

- All events described as Guillain-Barré syndrome will also be considered SAEs.

* Life-threatening adverse event. An adverse event 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 adverse event 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 (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE form and eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by an Independent Safety Monitor (ISM), the SMC (periodic review unless related), DMID, and the IRB.

9.2.5 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 study, regardless of the relationship to study product. AE/SAEs, abnormal clinical laboratory values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

9.3 Reporting Procedures

Solicited injection site and systemic reactogenicity events will be documented and reported from the time of each study vaccination through 8 days after each study vaccination.

Unsolicited non-serious AEs will be documented and reported from the time of each study vaccination through approximately 28 days after each study vaccination.

SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions will be documented and reported from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months (A/H7N9 arms) after the last study vaccination.

9.3.1 Serious Adverse Events

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 20814, 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 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 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.

9.3.2 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 adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. 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 study. Efforts will be made to follow all pregnancies reported during the course of this study 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 28 days after each study vaccination.

SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions will be collected, assessed, and followed from the time of the first study vaccination through resolution even if this extends beyond the study-reporting period (approximately Day 29 for A/H3N2v-vaccinated subjects and approximately 12 months after the last study vaccination for A/H7N9 $\pm$ AS03).

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 performed.

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:

- Any subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration.
- 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 generalized urticaria 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.
- Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product.
- Any subject develops a potentially immune-mediated medical condition after administration of study product.

This study will also be halted for SMC review/recommendation if, within 8 days after administration of each study vaccination, any of the following occurs:

- 10% or more of subjects (with a minimum of 2 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related injection site reaction. Ecchymosis (bruising), erythema (redness), and induration (hardness)/swelling will also be measured in mm but size will not be used as halting criteria.
- 10% or more of subjects (with a minimum of 2 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.

- 10% or more of subjects (with a minimum of 2 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction.

Grading scales for solicited local (injection site) 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 any study vaccination, then this study 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 study, 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 independent safety monitoring in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide an independent medical assessment and recommendation to DMID. The participating VTEU site will have an ISM with experience in infectious diseases or internal medicine, in close proximity to the participating VTEU site, and have the authority to readily access study participant records.

9.6.2 Safety Monitoring Committee (SMC)

Safety oversight will be conducted by a SMC that is an independent group of experts that monitors subject safety and advises DMID. The SMC members will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflict of interest related to this study. The SMC will consist of members with appropriate expertise to contribute to the interpretation of the data from this study.

The SMC will review study progress and participant, clinical, safety, and reactogenicity data at the following time points:

- Data review for safety at study specific time frames; at least annually
- After all 28-day post first study vaccination safety data are available.

- Ad hoc when a halting rule is met, for immediate concerns regarding observations during this study, or as needed.

The SMC will operate under the rules of a DMID-approved charter that will be approved at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. Procedures for SMC reviews/meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and participant, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. 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 in an unblinded fashion, and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this 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 study.

DMID or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if adverse events that meet the halting criteria are reported. 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 will be conducted to ensure that 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 study 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 compliance. Site monitors will have reasonable access to the participating VTEU site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with the site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Introduction

This study will use a systems biology approach to get a better understanding of how human immune cells respond to influenza A/H7N9 vaccination on the molecular level and how the addition of AS03 adjuvant modulates this response.

Specifically, this study will assess and characterize changes in gene expression and protein abundance in immune cells obtained from healthy adults after each of two doses of a monovalent inactivated influenza A/H7N9 virus vaccine manufactured by Sanofi Pasteur administered intramuscularly approximately 28 days apart at 3.75 mcg of HA (A/Shanghai/2/2013) per 0.5 mL [adjuvanted] or 0.25 mL [unadjuvanted] dose given with or without AS03 adjuvant manufactured by GSK. We will also compare these responses to one dose of unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur administered intramuscularly at 15 mcg of HA (A/Minnesota/11/2010) per 0.5 mL dose.

To elucidate the higher order organization of genes and proteins that differ between the three study arms, clusters with correlated response profiles, enriched functional modules and pathways as well as their placement within molecular networks will be determined. In addition, cytokine/chemokine concentrations well as serum anti-HA hemagglutination-inhibition (HAI) and neutralizing antibody concentrations will be measured. The goal is to correlate these measurements with responses on the molecular level to identify groups of genes and proteins that are associated with cytokine/chemokine production, seroconversion, or peak titer.

11.2 Study Hypotheses

This Phase II clinical study is not designed to test a specific hypothesis. Rather it is intended as an exploratory hypothesis-generating study to reveal new insights into how human immune cells respond to influenza A/H7N9 or A/H3N2 vaccination and how AS03 modulates responses to A/H7N9.

11.3 Study Outcome Measures

Refer to Section 3 for study outcome measures.

11.4 Sample Size Considerations

11.4.1 Study Design

This is a single center, randomized, partially-blinded, Phase II, small, targeted, prospective study in approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, designed to evaluate and compare the immunogenicity between an intramuscular monovalent inactivated influenza A/H7N9 virus vaccine manufactured by Sanofi Pasteur given with and without AS03 adjuvant manufactured by GlaxoSmithKline, and an intramuscular unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine manufactured by Sanofi Pasteur.

This study will use a standard and systems biology approach to assess the human early gene and protein signatures expressed at baseline (approximately Days -7 and 1), and at approximately Days 2, 4, and 8 (A/H3N2v-vaccinated subjects only) after the first study vaccination in each treatment arm as well as at approximately Days 29, 30, 32, and 36 in A/H7N9-vaccinated subjects only. Cellular immunogenicity (systems biology studies) data will be integrated with serologic immunogenicity (HAI and Neut antibody assays) and reactogenicity data to develop a systems model of the human immune response to unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine and monovalent inactivated influenza A/H7N9 virus vaccine given with and without AS03 adjuvant.

This study will use venous blood samples and subject data collected from a total of thirty vaccinated subjects randomly divided into three equal treatment arms. The first treatment arm (n=10) will be vaccinated with one dose of 15 µg of A/H3N2v HA. The second treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given with AS03 approximately 28 days apart. The third treatment arm (n=10) will be vaccinated with two doses of 3.75 µg of A/H7N9 HA given without AS03 adjuvant approximately 28 days apart. All study vaccinations will be administered intramuscularly.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study vaccination through 8 days after each study vaccination. Unsolicited non-serious adverse events (AEs) will be collected from the time of each study vaccination through approximately 28 days after each study vaccination. Serious adverse events (SAEs) and medically-attended adverse events (MAAEs) including new-onset chronic medical

conditions (NOCMCs) and potentially immune-mediated medical conditions (PIMMCs) will be collected from the time of the first study vaccination through approximately 1 month (A/H3N2v arm) and 12 months A/(H7N9 arms) after the last study vaccination.

Venous blood samples (approximately 90 mL) will be collected from subjects at approximately Days -7 and 1 (immediately prior to the first study vaccination), and at approximately Days 2, 4, and 8 (A/H3N2v-vaccinated subjects only), after the first study vaccination for systems biology studies (cytokine and chemokine levels, immune cell activation status, and whole transcriptome and proteome profiles of the major blood immune cells). Additionally, subjects in either treatment arm receiving A/H7N9 vaccine will have additional blood samples collected at approximately Days 29 (this will be collected immediately prior to the second study vaccination for subjects in either group receiving A/H7N9), 30, 32, and 36 after the first study vaccination for systems biology studies.

Serological assessment (hemagglutination inhibition (HAI) and neutralizing (Neut) antibody assays) will also be conducted on venous blood samples (approximately 10 mL) collected from subjects at Day 1 (immediately prior to the first study vaccination), and at approximately Days 29 (for A/H7N9-vaccinated subjects this will be collected immediately prior to the second study vaccination) and 57 (A/H7N9-vaccinated subjects only) after the first study vaccination.

We will quantify and characterize serum cytokine/chemokine levels, as well as transcriptomic and proteomic profiles from individual immune cell compartments, comparing this with standard serologic assessment to vaccine.

Urine samples (approximately 20 mL) will also be collected from subjects at approximately Days -7 1, 2, 4, 8 (A/H3N2v-vaccinated subjects only), 29, 30, 32, and 36 for future research. *Note: Days 29, 30, 32, and 36 will apply only to A/H7N9-vaccinated subjects.*

11.4.2 Study Population

The study population for this clinical study is approximately 30 healthy male and non-pregnant female subjects aged 18 to 49 years old, inclusive, who meet all eligibility criteria. The subjects will be recruited from the general population at the participating VTEU site that has substantial experience conducting influenza vaccine studies.

11.4.3 Sample Size

The sample size is not based on a formal hypothesis. Rather it is based on practical considerations with the goal of gathering enough information to learn more about immune system responses to the vaccine/adjuvant on the molecular level. DMID Protocol 10-0074,

entitled: *"A Randomized, Double-Blinded, Controlled, Phase I Study in Healthy Adults to Assess the Safety, Reactogenicity, and Immunogenicity of Intramuscular Subvirion Inactivated Monovalent Influenza A/H5N1 Virus Vaccine Administered With and Without AS03 Adjuvant: Standard & Systems Biology Analyses"*, showed that sample sizes in this range are sufficient to detect key differential signals and carry out systems biology analyses.

11.4.4 Planned Interim Analyses

Interim analyses would only be used to terminate this study 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. A SMC will be convened by DMID to review study progress and participant, clinical, safety, and reactogenicity data.

11.4.5 Interim Safety Review

An interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. 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 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. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this 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 study.

Additionally, this study will be monitored to determine if any of the halting rules described in Section 9.5 are met.

11.4.6 Interim Immunogenicity Review

An interim immunogenicity review is not planned.

11.5 Final Analysis Plan

Prior to database lock, a Statistical Analysis Plan that will outline in detail the planned statistical analyses for this study will be developed and finalized. In the following section an abbreviated version is provided.

11.5.1 Systems Biology Analyses

11.5.1.1 Transcriptomics (RNA-seq)

RNA-Seq data will be pre-processed by removing adapters and low-quality reads and mapping sequences to the latest human reference genome using splice-aware alignment software such as HISAT2.. Gene expression quantification will be carried out by using *Subread* against a common gene model reference. Systematic differences between samples will be normalized using the TMM method as implemented in the *edgeR* R package. Post normalization, genes that are known ribosomal, transfer, and mitochondrial RNA genes or genes located on the X or Y chromosomes will be excluded to avoid gender-specific effects Moderated log counts per million (LCPM) for the remaining genes will be determined using *edgeR*. LCPM will be used to identify lowly expressed genes, technical bias (e.g. batch effects or GC-content bias), and outlying samples. More specifically, Principal Component Analysis, non-metric multidimensional scaling, and correlation heatmaps based on LCPM will be carried out to identify potential global outliers and batch effects. Negative binomial models as implemented in *edgeR* will be used to determine differentially expressed genes for each endpoint and time point after removal of lowly expressed genes and outliers. If technical bias is detected and the effect is balanced across comparison groups, it will be accounted for by including a covariate as part of the negative binomial models. To compensate for testing multiple genes, p-values will be adjusted for by calculating false-discovery rates (FDR) based on the Benjamini-Hochberg procedure which controls the false positive rate among significantly differentially expressed genes. Genes with a FDR-value < 0.05 and a fold change of greater or equal to 1.5-fold (up or down regulation) will be deemed to be significantly differentially expressed (DE). Baseline responses (fold change in LCPM) of DE genes will be visualized using heatmaps. Unsupervised multiscale bootstrapping will be carried out to identify robust gene clusters with correlated responses (*pvclust* R package). Visualizations of fold change time trends for robust gene cluster will be provided. In addition, higher order organization of DE genes will be investigated using gene set enrichment analysis based on the latest MSigDB and KEGG databases accounting for gene length bias (*goseq* R package). Pathway maps color-coded by treatment effect will be provided for significantly enriched KEGG pathways. Experimentally determined protein-protein interaction networks including known Influenza A-host interactions centered around DE genes will be generated using data from the IntAct database and visualized using *Cytoscape*. Regularized canonical correlation

(*mixOmics* R package), linear and logistic regression models (*glmnet* R package) based on baseline fold change in LCPM will be carried out to determine gene responses that are correlated with cytokine/chemokine concentrations, peak titer, or seroconversion status, respectively.

11.5.1.2 Proteomics

A suitable global minimum reporter ion intensity threshold for peptide quantifications will be determined by exploring overall intensity distributions. Reported intensities below this threshold will be treated as missing. Next, peptide intensities will be divided by the reference channel ion intensities followed by $\log_2$ transformation of the ratio. Systematic differences between experiments will be accounted for by scaling channel ion intensities so that the channel medians of the $\log_2$ ratios are the same for all samples within a cell type. Log_2 protein reference ratios (LPR) are then calculated as the median of the normalized peptide $\log_2$ reference ratios.

Global patterns in LPR will be investigated using principal component analysis, hierarchical clustering, and multidimensional scaling to identify potential outlying samples and systematic effects. For these multivariate analyses, missing data will be imputed for each immune cell type using the k-nearest neighbors algorithm implemented in the impute R package. To accommodate smaller sample sizes, linear models as implemented in the *limma* R package will be used to identify differentially abundant proteins (DA) for each endpoint and time point. The advantage of the empirical Bayes approach as implemented in *limma* is that it leverages information across all proteins during the inference process thereby increasing statistical power. Proteins with a fold change that differs by at least 1.2 fold (up or down regulation) and individual p-value of <0.05 will be deemed to be significantly differentially abundant.

Baseline responses of DA proteins will be visualized using heatmaps. Higher order organization of DA proteins will be investigated using gene set enrichment analysis based on the latest MSigDB and KEGG databases using the Fisher's exact test implementation provided by the *goseq* R package. Pathway maps color-coded by treatment effect will be provided for significantly enriched KEGG pathways. Experimentally determined protein-protein interaction networks including known Influenza A-host interactions centered around DA proteins will be generated using data from the IntAct database and visualized using Cytoscape. Regularized canonical correlation (mixOmics R package), linear and logistic regression models (glmnet R package) based on baseline fold changes in LPR will be carried out to determine protein responses that are correlated with cytokine/chemokine concentrations, peak titer, or seroconversion status, respectively. The CD-HIT software will be used to determine protein families within the protein sequence database. This information will be integrated when presenting lists of DA proteins, heatmaps, and protein-interaction networks.

11.5.2 Serologic Immunogenicity Data (HAI and Neut Antibody Assays)

The 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) approximately 28 days following study vaccination will be summarized for each assay (HAI and Neut) and study arm using exact 95% confidence intervals. Comparisons between study arms will be carried out using a Fisher's exact test. Geometric Mean Titers (GMT) of serum HAI and Neut antibody and their 95% confidence intervals will be computed for Day -7 and 1 (baseline) and approximately 28 days following last study vaccination by transforming results to a logarithmic scale, assuming asymptotic normality conditions were satisfied on this scale, computing the mean and then converting back to the original scale. Differences between the treatments will be evaluated for Day 29 using two-sided t-test adjusting for unequal variance if necessary.

11.5.3 Cytokine/Chemokines

Cytokine/chemokine concentrations as well as their baseline fold changes will be summarized using minimum, Q1, median, Q3, maximum, and 95% CI of the median. The 95% CI of the median concentration and median fold change from baseline will be determined by using the bootstrap method and visualized using time trend plots. To identify cytokines/chemokines that show a differential response from baseline, a Wilcoxon signed-rank test will be carried out for each study arm and post-vaccination time point. Significant differences in fold changes between study arms for shared post-vaccination time points will be evaluated using a Wilcoxon rank-sum test.

11.5.4 Primary Clinical Study Report (CSR) Database Lock

Clinical, safety, and reactogenicity data through approximately 28 days after the last study vaccination will represent the primary clinical database for this study. Once the last subject completes the visit that occurs approximately 28 days after the last study vaccination, the primary clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, and available immunogenicity data may be performed by the SDCC after the primary clinical database is locked. These analyses will be made available to the sponsor for planning subsequent studies and to the clinical investigators for publication. These analyses will not be used to make any decisions concerning the conduct of this study. As it is anticipated subjects will remain in the long-term, tertiary safety follow-up at the time of these analyses, the presentation of these data will be in a format that prevents inadvertent unblinding of the sponsor or clinical investigators to any individual subject's treatment assignment or immune response data. When it is not possible to display the data by treatment arm and maintain the blind for the

clinical investigators and sponsor, such as the presence of extreme values or noteworthy AEs, a presentation by treatment arm will not be provided until after the long-term, tertiary safety follow-up is complete.

Serologic immunogenicity data from HAI and Neut antibody assays and processed cellular immunogenicity data from systems biology studies may be analyzed as results are available once the primary clinical database is locked. These analyses will be considered the final results for this study and included as part of the clinical study report (CSR).

The primary CSR will be completed when all primary and secondary endpoint data are available. Any available data from the long-term, tertiary safety follow-up of applicable subjects may also be included. Additional data from the long-term, tertiary safety follow-up of applicable subjects, exploratory correlation analyses, and data from the proteomics portion of the study will be included in one or more addenda to the CSR.

A formal statistical analysis plan will be developed and finalized prior to database lock.

11.5.5 Analysis Populations

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

The intent-to-treat (ITT) population includes all subjects who received at least one dose of study vaccine and contributed both pre- and at least one post-study vaccination blood samples for serological assessment (HAI or Neut antibody assays) for which valid results were reported.

The per protocol (PP) population includes all subjects in the ITT 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,
    - Receipt of non-study licensed live vaccine within 30 days before or after each study vaccination,
    - Receipt of non-study licensed inactivated vaccine within 14 days before or after each study vaccination,
    - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days before or after each study vaccination.

- Data from any visit that occurs substantially out of window.

11.5.6 Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population. These include reactogenicity following each study vaccination, unsolicited adverse events, and SAEs/MAAEs (including new-onset chronic medical conditions and potentially immune-mediated medical conditions. These safety data will be analyzed, in an exploratory manner, in the context of transcriptomic/proteomic changes.

11.5.7 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 DATA COLLECTION FORMS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating VTEU site will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. The participating VTEU 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 study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or 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 study. Source data are all information, original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study.

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

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating VTEU site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigators 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 investigators 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 VTEU site for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site principal investigators will ensure that this study is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the 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 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site principal investigators' institution will hold a current Federalwide Assurance (FWA) issued by Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Prior to enrollment of subjects into this study, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this study and a copy will be provided to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site principal investigator for submission to the IRB.

14.3 Informed Consent Process

14.3.1 Informed Consent

The site principal investigators will choose subjects in accordance with the eligibility criteria detailed in Section 5.1. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB. 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.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. Before any study procedures are performed, including pre-screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products. This will include the nature, risks and possible benefits of this study, alternate therapies, any known AEs, the investigational status of the study interventions/products, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum and urine samples. Subjects will be allowed sufficient time to consider participation in this research study, after having the nature, risks and possible benefits of this study explained to them, and have the opportunity to discuss this study with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits will be given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain this research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures specifically for this study, including determining eligibility and administering study product.

By signing the informed consent form, subjects agree to complete all study procedures required by this study, unless the subject withdraws voluntarily, or is withdrawn or terminated from this study for any reason. The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this study.

DMID will provide the site principal investigators, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational products. This new information will be communicated by the site principal investigators to subjects who consent to participate in this study in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

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

This study will be inclusive of all adults who meet the Subject Inclusion Criteria (see Section 5.1.1) and do not meet the Subject Exclusion Criteria (see Section 5.1.2), regardless of religion, sex, or ethnic background. Should the outcome of this study be deemed acceptable, additional studies may be initiated including those in other populations.

It is unknown if the monovalent inactivated influenza A/H7N9 virus vaccine with or without AS03 adjuvant or unadjuvanted monovalent inactivated influenza A/H3N2v virus vaccine poses any risks to an unborn child. As of November 22, 2015 (per the most current version of the manufacturer's IB), the available data for women who become pregnant during clinical trials of AS03-adjuvanted (pre) pandemic influenza vaccines do not suggest any causal relationship between adverse pregnancy outcomes and receipt of an AS03-adjuvanted vaccine. Women of childbearing potential who are not surgically sterile via tubal sterilization, bilateral oophorectomy or hysterectomy, or who are not postmenopausal for ≥1 year must agree to practice highly effective contraception that may include, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms that contain spermicide or with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 60 days after their last study vaccination. A highly effective method of contraception is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all women of childbearing potential will be required to have a negative urine or serum pregnancy test within 24 hours prior to each study vaccination. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Children will not be included in this study.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the site principal investigators, other study personnel, the sponsor, and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. Subjects will have code numbers and will not be identified by name.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning this study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the participating VTEU site as part of this study (other than a subject's medical records) will be kept confidential by the site principal investigators and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting this study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this study; (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigators. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The participating VTEU site will permit access to such records.

14.6 Study Discontinuation

If this study is discontinued, subjects who have signed the informed consent form and are randomized and vaccinated will continue to be followed for safety for the duration of this study. No further study vaccinations will be administered.

14.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this study.

Subjects may be compensated for their participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the participating VTEU site and the site principal investigators that an injury occurred to a subject as a direct result of the tests or treatments that are done for this study, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating VTEU site, such as giving emergency medications to stop immediate allergic reactions to the study vaccines. No financial

compensation will be provided to the subject by the participating VTEU site for any injury suffered due to participation in this study.

14.8 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining serum 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. Urine samples (approximately 20 mL) will also be collected from subjects for future research.

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. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on the samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality.

There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of this study. The subject's decision can be changed at any time by notifying the study doctors or nurses in writing; however, if a subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

15 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the SDCC to record and maintain data for each subject enrolled in this study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, 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.

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms 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

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

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

The SDCC 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), reactogenicity, serologic immunogenicity (HAI and Neut antibody assays), and processed cellular immunogenicity (systems biology studies) data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks,

to identify data that appear inconsistent, incomplete, or inaccurate. Clinical and reactogenicity data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., reactogenicity, serologic immunogenicity (HAI and Neut antibody assays), and processed cellular immunogenicity (systems biology studies) data).

15.4 Timing/Reports

Clinical, safety, and reactogenicity data through approximately 28 days after the last study vaccination will represent the primary clinical database for this study. Once the last subject completes the visit that occurs approximately 28 days after the last study vaccination, the primary clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, and available immunogenicity data may be performed by the SDCC after the primary clinical database is locked. These analyses will be made available to the sponsor for planning subsequent studies and to the clinical investigators for publication. These analyses will not be used to make any decisions concerning the conduct of this study. As it is anticipated subjects will remain in the long-term, tertiary safety follow-up at the time of these analyses, the presentation of these data will be in a format that prevents inadvertent unblinding of the sponsor or clinical investigators to any individual subject's treatment assignment or immune response data. When it is not possible to display the data by treatment arm and maintain the blind for the clinical investigators and sponsor, such as the presence of extreme values or noteworthy AEs, a presentation by treatment arm will not be provided until after the long-term, tertiary safety follow-up is complete.

Serologic immunogenicity data from HAI and Neut antibody assays and processed cellular immunogenicity data from systems biology studies may be analyzed as results are available once the primary clinical database is locked. These analyses will be considered the final results for this study and included as part of the clinical study report (CSR).

The primary CSR will be completed when all primary and secondary endpoint data are available. Any available data from the long-term, tertiary safety follow-up of applicable subjects may also be included. Additional data from the long-term, tertiary safety follow-up of applicable subjects, exploratory correlation analyses, and data from the proteomics portion of the study will be included in one or more addenda to the CSR.

Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety 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 records and reports, including, but not limited to, eCRFs, source documents, informed consent forms, and study drug disposition records, shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and FDA has been so notified. The participating VTEU site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigators, or other study 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 investigators and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC's AdvantageEDC^{SM}.

All protocol 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/IEC per its guidelines. The site principal investigator and other study 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

Following completion of this clinical trial, the lead principal investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

It is the responsibility of DMID to register this clinical trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For clinical trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the clinical trial for publication.

For clinical trials in which DMID is not the IND/IDE sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to register the clinical trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

Refer to:

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

*Journal Citation: De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004; 351:1250-1.

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APPENDICES

APPENDIX A: LIST OF POTENTIALLY IMMUNE-MEDIATED MEDICAL CONDITIONS

APPENDIX B: SCHEDULE OF EVENTS

APPENDIX A: LIST OF POTENTIALLY IMMUNE-MEDIATED MEDICAL CONDITIONS

(also known as Adverse Events of Special Interest (AESIs))

Gastrointestinal disorders
• Celiac disease
• Crohn's disease
• Ulcerative colitis
• Ulcerative proctitis

Liver disorders
• Autoimmune cholangitis
• Autoimmune hepatitis
• Primary biliary cirrhosis
• Primary sclerosing cholangitis

Metabolic diseases
• Addison's disease
• Autoimmune thyroiditis (including Hashimoto thyroiditis)
• Diabetes mellitus type I
• Grave's or Basedow's disease

Musculoskeletal disorders
• Antisynthetase syndrome
• Dermatomyositis
• Juvenile chronic arthritis (including Still's disease)
• Mixed connective tissue disorder
• Polymyalgia rheumatic
• Polymyositis
• Psoriatic arthropathy
• Relapsing polychondritis
• Rheumatoid arthritis
• Scleroderma, including diffuse systemic form and CREST syndrome
• Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
• Systemic lupus erythematosus
• Systemic sclerosis

Neuroinflammatory disorders
• Acute disseminated encephalomyelitis, including site specific variants (e.g., non-infectious encephalitis, encephalomyelitis,myelitis, myeloradiculomyelitis)
• Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy)

• Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
• Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy
• Multiple sclerosis
• Narcolepsy
• Optic neuritis
• Transverse myelitis
• Myasthenia gravis, including Eaton-Lambert syndrome

Skin disorders
• Alopecia areata
• Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis
• Cutaneous lupus erythematosus
• Erythema nodosum
• Morphoea
• Lichen planus
• Psoriasis
• Sweet's syndrome
• Vitiligo

Vasculitides
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
• Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis,Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

Others
• Antiphospholipid syndrome
• Autoimmune hemolytic anemia
• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
• Autoimmune myocarditis/cardiomyopathy
• Autoimmune thrombocytopenia
• Goodpasture syndrome
• Idiopathic pulmonary fibrosis
• Pernicious anemia
• Raynaud's phenomenon
• Sarcoidosis

- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis

APPENDIX B: SCHEDULE OF EVENTS

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04Ω**	**V05**	**V06~**	**V07~**	**V08~**	**V09~**	**V10~***	**V11~***	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post first study vaccination	**Screening D-7 (D-14 to D-5)**	**Enrollment and Dose 1 D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	***D30***	***D32***	***D36***	***D57***	***D209***	***D394***		
Study Day post second study vaccination						**Dose 2~ D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	**D181±14d**	**D366±14d**		
Obtain Informed Consent∞	X	X¬												
Collect Demographic Information	X													
Review Eligibility Criteria	X	X†¬				X~†								
Medical History@	X	X†¬	X	X	X[3]	X†	X	X	X	X			X	X (if indicated)
Concomitant Medications!	X√	X†¬	X	X	X	X†	X	X	X	X			X (if prior to 28 days after last study vaccination)	X (if prior to 28 days after last study vaccination)
Vital Signs$ (Oral Temperature%, Pulse, and BP)	X	X†				X~†							X (may be obtained if indicated)	X (may be obtained if indicated)
Height and Weight		X†												
Targeted Physical Examination	(X)	(X)†	(X)	(X)	(X)[3]	(X)†	(X)	(X)	(X)	(X)			(X)	(X)
Urine or Serum Pregnancy Test	X^	X^				X~^								
Venous Blood Collection for ESR[2]	X													

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04[Ω]**	**V05**	**V06~**	**V07~**	**V08~**	**V09~**	**V10~***	**V11~***	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post first study vaccination	**Screening D-7 (D-14 to D-5)**	**Enrollment and Dose 1 D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	***D30***	***D32***	***D36***	***D57***	***D209***	***D394***		
Study Day post second study vaccination						**Dose 2~ D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	**D181±14d**	**D366±14d**		
Venous Blood Collection for HAI and Neut Antibody Assays		X[†]				X[†]				X			X (if prior to 28 days after last study vaccination)	
Venous Blood Collection for Systems Biology Studies[1]	X	X[†]	X	X	X[3]	X~[†]	X	X	X				X (if prior to 28 days after last study vaccination)	
Urine Collection for Future Research	X	X[†]	X	X	X[3]	X~[†]	X	X	X				X (if prior to 28 days after last study vaccination)	
Enrollment in AdvantageEDC[SM] and Randomization		X[†]												
Pre-Administration Reactogenicity Assessments		X[†]				X~[†]								
Study Vaccination		X				X~								
20-minute Evaluation After Study Vaccination		X				X~								

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04[Ω]**	**V05**	**V06~**	**V07~**	**V08~**	**V09~**	**V10~***	**V11~***	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post first study vaccination	**Screening D-7 (D-14 to D-5)**	**Enrollment and Dose 1 D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	***D30***	***D32***	***D36***	***D57***	***D209***	***D394***		
Study Day post second study vaccination						**Dose 2~ D1**	**D2+1d**	**D4+1d**	**D8+2d**	**D29±2d**	**D181±14d**	**D366±14d**		
Examine Vaccination Site		X	X	X	X[3]	X~	X	X	X				X (if within 8 days after last study vaccination)	X (if within 8 days after last study vaccination)
Post-Administration Reactogenicity Assessments		X				X~							X (if within 8 days after last study vaccination)	X (if within 8 days after last study vaccination)
Distribute Memory Aid and Study-Related Materials		X				X~								
Review Memory Aid			X	X	X		X	X	X				X (if within 8 days after last study vaccination)	X (if within 8 days after last study vaccination)
AE/SAE Assessment		X[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X	X[#]	X[#]	X**(AEs if prior to 28 days after last study vaccination)	X** (AEs if prior to 28 days after last study vaccination)

* Phone call/electronic communication (e.g., email, text message) assessment.
∞ Prior to study procedures.
† Prior to study vaccination.
¬ Review/confirm information or activity in subjects previously consented and screened.

@ Complete medical history will be obtained by interview of the subjects at the screening visit and will be updated on Day 1 prior to the first study vaccination and interim medical history will be obtained by interview of the subjects at follow-up visits after the first study vaccination.

! Including solicitation through Visit 09 for receipt of non-study influenza vaccines.

√ All current medications and medications taken within 60 days prior to signing the informed consent form. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination.

$ Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline.

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

() Targeted physical examination if indicated based on review of complete or interim medical history.

^ Will be performed on all women 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.

1 Including cytokine and chemokine levels.

~ A/H7N9-vaccinated subjects only.

& Inclusive of reactogenicity assessments performed on the day of each study vaccination through 8 days after each study vaccination.

Assessment of AE/SAE limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditions.

** Assessment of AE/SAE limited to SAEs and MAAEs including new-onset chronic medical conditions and potentially immune-mediated medical conditionsif after 28 days following the last study vaccination.

2 To be performed locally by the site. The ESR value must be confirmed as <30 mm/hr prior to randomization and first study vaccination.

3 A/H3N2v-vaccinated subjects only.

Ω Visit 04 is an in-clinic assessment for A/H3N2v-vaccinated subjects and a phone call/electronic communication (e.g., email, text message) assessment for A/H7N9-vaccinated subjects.