Biomedical Advanced Research and Development Authority

BARDA BP-I-16-005

Randomized, Double-Blinded, Phase 2 Study to Assess Safety and Immunogenicity of Homologous and Heterologous Prime-Boost Vaccination Strategies With Stockpiled Inactivated Monovalent Influenza A(H5) Vaccines Administered Intramuscularly With Either AS03 or MF59® as Adjuvant

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Statistical Analysis Plan

Version *1.2*

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Upon review of this document, the undersigned approves the statistical analysis plan. The analysis [redacted]

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Table of Contents

List of Tables

List of Abbreviations

AE	Adverse Event
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body Mass Index
BP	Blood Pressure
CIs	Confidence Intervals
CSR	Clinical Study Report
cm	centimeters
eCRF	electronic Case Report Form
FAS	Full Analysis Set
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IFAS	Immunogenicity-Full Analysis Set
IM	Intramuscularly
IP	Investigational Product
IPPS	Immunogenicity Per-Protocol Set
IRT	Interactive Response Technology
IVRS	Interactive voice Response System
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NPIVS	National Pre-Pandemic Influenza Vaccine Stockpile
PBMC	Peripheral Blood Mononuclear Cell
PIMMC	Potentially Immune-mediated Medical Conditions
PT	Preferred Term
PPD	Pharmaceutical Product Development
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SOC	System Organ Class
SPR	Seroprotection Rate
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organization

1 Introduction

In order to prepare and respond effectively to future influenza pandemic threats, the Biomedical Advanced Research and Development Authority (BARDA) within the Office of the Assistant Secretary for Preparedness and Response, has established and maintains the National Pre-Pandemic Influenza Vaccine Stockpile (NPIVS) that comprises bulk adjuvants, pre-pandemic antigens, and final containers of vaccines that were manufactured from candidate vaccine viruses from representative clades of H5 influenza A viruses (H5 influenza viruses). Production issues and other factors make it difficult to estimate how long it would take during a pandemic emergency to produce enough doses of vaccine exactly matching the antigenic properties of the virus, to protect the United States population. Therefore, it is important to develop potential strategies for immediate deployment of stockpiled H5 influenza virus vaccines as an interim intervention in response to a pandemic emergency. Previous studies suggest that heterologous prime and boost vaccination regimens (i.e., different HA antigens used for prime and boost vaccinations) may be superior to a homologous prime and boost (i.e., same HA antigens used for prime and boost vaccinations) vaccination regimen in generating cross-reactive immune responses to antigenically divergent influenza viruses. Furthermore, there is some evidence suggesting that when compared to a homologous prime-boost vaccination regimen, a heterologous prime-boost vaccination regimen including adjuvant may broaden and enhance cross-reactive hemagglutination inhibition (HAI) antibody responses to a panel of antigenically divergent H5 influenza viruses (unpublished data).

Influenza vaccines induce antibodies against the viral HA antigen in the vaccine, thereby blocking viral attachment to human respiratory epithelial cells. In some human challenge studies of other influenza viruses, antibody titers of at least 1:40 have been associated with protection from influenza infection in up to 50% of subjects.

Sanofi Pasteur Influenza A/Vietnam/1203/2004 (H5N1), A/Indonesia/05/2005 (H5N1), and A/bar-headed goose/Qinghai Lake/1A/2005 (H5N1) vaccines contain 30 mcg/mL HA from SJRG-161052, Indo5/PR8-RG2, and SJRG-163222 candidate vaccine viruses respectively, in sodium phosphate buffered isotonic saline. These monovalent vaccines also contain gelatin (0.05%), trace amounts of formaldehyde (≤200 mcg/mL), polyethylene glycol p-isooctylphenyl ether (≤0.05%), and sucrose (≤2.0%). In addition, thimerosal at a concentration of approximately 0.01% was added as a preservative (Fluzone Quadrivalent [Influenza Vaccine] 2016).

Seqirus (formerly Novartis) A/duck/Bangladesh/19097/2013 H5N1 vaccine contains 30 mcg/mL HA from SJ007 candidate vaccine virus in sodium phosphate buffered isotonic saline. Each 0.5 mL vial of monovalent vaccine may also contain residual amounts of Madin Darby canine kidney cell protein (≤8.4 mcg), protein other than HA (≤160 mcg), Madin Darby canine kidney cell DNA (≤10 ng), polysorbate 80 (≤1500 mcg), cetyltrimethylammonium bromide (≤18 mcg), and β–propiolactone (≤0.5 mg) (Flucelvax Quadrivalent [Influenza Vaccine] 2016).

Seqirus (formerly bioCSL) A/gyrfalcon/Washington/41088-6/2014 (H5N8) vaccine contains 30 mcg/mL HA from IDCDC-RG43A candidate vaccine virus in isotonic phosphate buffer. Each 0.5 mL vial of monovalent vaccine may also contain trace amounts of sodium taurodeoxycholate (≤10 ppm), ovalbumin (<1 mcg), neomycin sulfate (≤0.2 pg), polymyxin B (≤0.03 pg), and beta-propiolactone (≤25 ng) (Afluria 2010).

The adjuvant MF59® is an oil-in-water emulsion composed of squalene and a buffer, which has been developed to enhance the immunogenicity of purified subunit antigens. There is evidence showing that such vaccine antigens, when administered with MF59, are safe and generally tolerated.

The AS03 adjuvant is composed of squalene, DL-α-tocopherol, and polysorbate 80. AS03 is approved for human use as the adjuvant component of the Influenza H5N1 Virus Monovalent Vaccine, Adjuvanted (Influenza A (H5N1) Virus Monovalent Vaccine 2013). There are no major safety concerns associated with the use of AS03 adjuvants. However, there is epidemiological evidence of an association between influenza vaccine adjuvanted with AS03 and narcolepsy.

This statistical analysis plan (SAP) describes the analyses and data presentations for the two planned interim analyses, final clinical study report (CSR) and the addendum to the CSR based upon study protocol version 1.2 dated January 26, 2018. It includes the definitions of analysis sets and derived variables and describes statistical methods for the analyses of safety and immunogenicity. The main purpose of this study is to assess the ability of H5 influenza virus vaccines and adjuvants present in the NPIVS to generate an immune response to homologous and to antigenically distant heterologous H5 influenza virus strains. Therefore, there will be no formal statistical comparison between any study groups planned for the primary and secondary objectives.

Analyses and data presentation for the CSR will include all the data up to Day 142 for the 2-dose vaccination series and Day 262 for the 3-dose vaccination series. Study disposition, concomitant medications/vaccinations, MAAEs and SAEs will be collected through end of the study and the corresponding summary tables/listings will be updated and presented in the addendum to the CSR once the study is complete.

2 Objectives

2.1 Primary Objectives

2.1.1 Primary Safety Objective

- To assess the safety and reactogenicity for 8 days postvaccination, inclusive of the vaccination Day (Day 1 through Day 8 and Day 22 through Day 29), of a 2-dose vaccination series using homologous monovalent inactivated H5 influenza virus vaccine or antigenically distant heterologous H5 influenza virus vaccines administered intramuscularly (IM) with AS03 or MF59 adjuvant on Days 1 and 22 (henceforth referred to as “prime-boost”), as determined by solicited local and systemic reactogenicity symptoms.
- To assess the safety and reactogenicity for 8 days postvaccination, inclusive of the vaccination Day (Day 1 through Day 8, Day 22 through Day 29, and Day 142 through Day 149), of a 3-dose vaccination series using homologous monovalent inactivated H5 influenza virus vaccine administered IM with AS03 or MF59 adjuvant on Days 1 and 22, and a third dose 120 days after the second dose with an antigenically heterologous H5 influenza virus vaccine administered IM with AS03 or MF59 adjuvant on Day 142 (henceforth referred to as “prime-prime-boost”), as determined by solicited local and systemic reactogenicity symptoms.

2.1.2 Primary Immunogenicity Objective

- To assess the serum HAI antibody seroprotection rate (SPR) against strains contained in the study vaccines on Day 43 of the prime-boost vaccination series administered IM with AS03 or MF59 adjuvant on Days 1 and 22.
- To assess the serum HAI antibody SPR against strains contained in the study vaccines on Day 163 of the prime-prime-boost vaccination series administered IM with AS03 or MF59 adjuvant on Days 1, 22, and 142.

2.2 Secondary Objectives

2.2.1 Secondary Safety Objectives

- To assess the occurrence of serious adverse events (SAEs), medically attended adverse events (MAAEs), and potentially immune-mediated medical conditions (PIMMCs; see Appendix 2, Section 14.2) in the 12 treatment groups through 12 months after the last dose of study vaccine.
- To assess the occurrence of unsolicited adverse events for 21 days after each dose of study vaccine.

2.2.2 Secondary Immunogenicity Objectives

- To assess the serum HAI antibody titers, SPRs, and seroconversion rates (SCRs) against each vaccine strain in the study through Day 142 of the prime-boost vaccination series.
- To assess the serum HAI antibody titers, SPRs, and SCRs against each vaccine strain in the study through Day 262 of the prime-prime-boost vaccination series.
- To assess the serum microneutralization (MN) antibody titers and SCRs against each vaccine strain in the study through Day 142 of the prime-boost vaccination series.
- To assess the serum MN antibody titers and SCRs against each vaccine strain in the study through Day 262 of the prime-prime-boost vaccination series.

3 Investigational Plan

3.1 Overall Study Design and Plan

This is a randomized, double-blinded, Phase 2 study to assess the safety and immunogenicity of a homologous and heterologous prime-boost or prime-prime-boost series with inactivated monovalent influenza H5 vaccines stored in the NPIVS, administered IM with either AS03 or MF59 adjuvant in healthy males and nonpregnant females, aged 18 to 49 years, inclusive.

Screening will occur within a 12-Day period prior to 2 days before randomization. Approximately 720 subjects will be equally and randomly assigned to one of 12 study groups within each study site. Study staff not involved in investigational product (IP) preparation and administration will be blinded to unique group assignment. Two doses of adjuvanted vaccine separated by 21 days (Groups A-D, G-J) or 2 doses of adjuvanted vaccine separated by 21 days followed by a third dose of adjuvanted vaccine 120 days following the second dose (Groups E, F, K and L) will be administered. All administered vaccines include HA antigen from the NPIVS.

Approximately half of enrolled subjects per study group will be selected to provide a blood sample for peripheral blood mononuclear cell collection.

Investigational product treatment arms are detailed in Table 3-1.

Table 3-1 Treatment Arms

Study Group	Dose #1 (Day 1) Strain Details (Manufacturer)	Dose #2 (Day 22) Strain Details (Manufacturer)	Dose #3 (Day 142) Strain Details (Manufacturer)	Adjuvant
A	A/Vietnam/1203/2004 H5N1 (Sanofi Pasteur)	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	N/A	AS03
B	A/Indonesia/05/2005 H5N1 (Sanofi Pasteur)			
C	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)			
D	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/Indonesia/05/2005 H5N1 (Sanofi Pasteur)		
E	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)	A/barheaded goose/Qinghai Lake/1A/2005 (H5N1) (Sanofi Pasteur)	
F	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/barheaded goose/Qinghai Lake/1A/2005 (H5N1) (Sanofi Pasteur)	
G	A/Vietnam/1203/2004 H5N1 (Sanofi Pasteur)	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	N/A	MF59
H	A/Indonesia/05/2005 H5N1 (Sanofi Pasteur)			
I	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)			
J	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/Indonesia/05/2005 H5N1 (Sanofi Pasteur)		
K	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)	A/duck/Bangladesh/19097/2013 H5N1 (Seqirus)	A/barheaded goose/Qinghai Lake/1A/2005 (H5N1) (Sanofi Pasteur)	
L	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus)	A/barheaded goose/Qinghai Lake/1A/2005 (H5N1) (Sanofi Pasteur)	

Abbreviation: N/A, not applicable.

Data will be analyzed by Pharmaceutical Product Development (PPD) according to the data analysis Section 7 of the study protocol. Important information is given in the following sections and details are provided, as applicable, in this document. The schedule of events is shown in Table 14-1 and Table 14-2 in Appendix 1.

3.2 Study Endpoints

3.2.1 Primary Endpoints

3.2.1.1 Primary Safety Endpoint

Occurrence of mild, moderate, or severe solicited local and systemic reactogenicity symptoms during the 8 days after each vaccination, inclusive of the vaccination day.

3.2.1.2 Primary Immunogenicity Endpoints

SPR (defined as the proportion of subjects achieving a serum HAI titer of at least 1:40 against influenza antigen) against strains contained in the study vaccines on Day 43 for Groups A-D and G-J and on Day 163 for Groups E, F, K and L.

3.2.2 Secondary Endpoints

3.2.2.1 Secondary Safety Endpoints

- Occurrence of SAEs, MAAEs, and PIMMCs in 12 treatment groups through 12 months after the last dose of study vaccine
- Occurrence of any AEs leading to study withdrawal
- Frequency, relatedness, and severity of unsolicited AEs for 21 days following each vaccination
- Occurrence of clinical safety laboratory abnormalities at all collected time points
- Occurrence of vital sign abnormalities at all collected time points

3.2.2.2 Secondary Immunogenicity Endpoints

- Serum HAI and MN antibody titers at all collected time points
- SPR of serum HAI antibody at collected time points
- Seroconversion rate (defined as proportion of subjects achieving either a prevaccination antibody titer of <1:10 and a postvaccination titer of at least 1:40 or a prevaccination antibody titer of at least 1:10 and a 4-fold or greater increase of postvaccination antibody titer; if antibody titer is undetectable, it will be assigned a value of half the lower limit of detection) at all collected postvaccination time points

3.3 Study Groups

This is a double-blinded study with 12 study groups (i.e. treatment groups). The summary will be based on each of the 2 adjuvants (summary table includes 6 treatment arms in one page for each adjuvant). For subjects missed the second and/or third vaccinations, the randomized treatment groups will be used for summarizing these subjects. For a complete list of study groups and corresponding vaccination and adjuvants schemes refer to Table 3-1.

4 General Statistical Considerations

For continuous variables, descriptive statistics will typically include the number of subjects, mean, standard deviation (SD), 25th percentile (Q1), median, 75th percentile (Q3), minimum, and maximum. For categorical variables, descriptive statistics will typically include the number and percentage of subjects in each category. Unless otherwise noted, data from all sites will be combined in the computation of these descriptive statistics.

In statistical summaries, mean, median, Q1, and Q3 will be displayed to one more decimal place than the original value; minimum and maximum will keep the same number of decimal places as the original value; standard deviation will be displayed to two more decimal places than the original value; percentages will generally be reported to one decimal place.

Computation for all the results will be performed using SAS® version 9.3 or higher computer software package.

Baseline

Unless otherwise specified, baseline will be defined as the last non-missing measurement prior to the first vaccination.

Study Day

Study Day will be calculated as event/assessment date - first dose date of IP if the assessment occurs before the first dose; Study Day will be calculated as event/assessment date - first dose date of IP + 1 Day if the assessment occurs on or after the first dose.

Subject Counts in Table Headers

The subject counts (N's) for each study group in the table header will equal the number of subjects who receive the actual treatment as the study group displayed unless otherwise specified. All other subjects who receive any vaccination different from group A-L regimen will be included in the listings and overall column (per adjuvant) if available. Overall column will be included in the tables based on randomized treatment only.

Safety Data Reporting Periods

The safety data reporting periods are shown in Table 4-1.

Table 4-1 Safety Data Reporting Periods

Type of Event	Collection Period: Study Days
Solicited AEs	Days 1-8 Post each vaccination
Unsolicited AEs [a]	Days 1-21 Post each vaccination
SAEs, MAAEs, and PIMMCs	Entire Study Period [b]
Pregnancies	Entire Study Period [c]

Abbreviations: AE, adverse event; MAAE, medically attended adverse event; PIMMC, potentially immune-mediated medical condition; SAE, serious adverse event.

[a] Unsolicited AEs will be collected through the end of the study visit window for each AE collection period (i.e., 21 days after each vaccination ±3-Day window = 21 ±3 days).

[b] The entire study period is from the time a subject receives the first study injection through the end-of-study visit. If the investigator becomes aware of any related SAE or related MAAE after exit from or completion of the study, he or she is obligated to report this event.

[c] Pregnancies that occur during a subject's participation in the study will be followed until resolution and 2 weeks after live birth even if the pregnancy ends after the end-of-study visit.

Subject Grouping in Data Listings

In general, subjects will be grouped or sorted with other subjects who belong to the same study group as treated. However, in the event that a subject incorrectly receives doses of IP different from group A-L regimen, the subject will be grouped or sorted in separate categories based on actual vaccines received.

Missing Data

Missing data will not be imputed unless otherwise specified.

Adverse events and concomitant medications with missing start dates will have their start dates imputed.

If a start date is completely missing, then the first dose date of IP will be used.

If both Day and month are missing but year is available (--/---/YYYY), then the date will be imputed as follows:

- If the year is less than the year the IPs were administered, then the start date will be imputed as Jul 1st.
- If the year is equal to the year the IPs were administered, then the start date will be imputed as the first dose date of IP.
- If the year is greater than the year the IPs were administered, then Jan 1st of the year will be used.

If only Day is missing (--/MMM/YYYY), then the following rules will be applied relative to the year that the IPs were given:

- If the year is equal to the year that the IPs were administered, then
    - If the start month is equal to the month that the first dose of IP was administered, then the first dose date of IP will be used.
    - If the start month is equal to the month that the second dose of IP was administered, then the second dose date of IP will be used. In the case that the first and second

doses of IPs were administered in the same month, the first dose date of IP will be used.

- If the start month is equal to the month that the third dose of IP was administered, then the third dose date of IP will be used.
- If the start month is greater than the month that the last dose of IP was administered, or is between two doses of IP but not equal to either of the months the dose of IP was administered, then the 1st Day of the month will be used.

- If the year is greater than the year that the IPs were administered, then the 1st of the month will be used.

4.1 Sample Size

Approximately 720 subjects with 60 subjects per study group will be enrolled in the study. Approximately half of enrolled subjects will be selected to provide a blood sample for PBMC collection.

The number of subjects proposed to be enrolled in this protocol is based upon previous experience with similar studies such as the Mix and Match Studies (NCT 01317745 and NCT 01217758) performed by Division of Microbiology and Infectious Diseases/National Institute of Allergy and Infectious Diseases. These prior studies had study groups of similar size that proved to be sufficient to allow the collection of meaningful data, especially with respect to acute solicited AEs and antibody results. While the current study is not designed to test specific hypotheses, Table 4-2 and Table 4-3 indicate the power of the proposed sample size to detect a range of common, immediate postvaccination elicited safety events as well as to meet the success criteria for SPR.

Table 4-2 Safety - Power for Detecting at Least 1 Serious Adverse Event (SAE) or Medically-Attended Adverse Event (MAAE) or a Grade 3 Solicited Adverse Event (AE)

True Event Rate (%)	Power (%) N = 60	Power (%) N = 120
0.1	5.8	11.3
0.5	26.0	45.2
1	45.3	70.1
2	70.2	91.2
3	83.9	97.4
4	91.4	99.3
5	95.4	99.8

Note: The exact method was used for the calculations in this table.

Table 4-3 Immunogenicity - Power for Meeting Success Criteria of Seroprotection Rate (SPR)

Success Criteria (%)	Proportion of Subjects Achieving a Serum HAI Antibody Titer of at Least 1:40 for the Testing Vaccine (%)	Power (%) N = 60	Power (%) N = 120
70	90	98.5	>99.9
	85	81.9	99.0
	80	44.9	79.0

4.2 Randomization, Stratification, and Blinding

4.2.1 Randomization

Subjects will be randomly assigned to 1 of 12 study groups stratified by site. An Interactive Response Technology (IRT) will be used to administer the randomization schedule centrally. Biostatistics will generate the randomization schedule using SAS® software Version 9.3 or later (SAS Institute Inc, Cary, North Carolina) for IRT, which will link sequential subject randomization numbers to treatment codes. The randomization schedule will be created by the dedicated randomization team, stored in a separate project area, and will be blinded to the project team.

4.2.2 Stratification

Randomization is stratified by site to balance treatment assignments over the course of the trial within each site.

4.2.3 Blinding

This is a double-blind study. All IP will be prepared and administered by an unblinded study staff member. IP accountability will be monitored by a separate unblinded monitor. All other persons involved in the study will be blinded to study group assignment, including the study staff observing the subject after vaccination and the subject. Until the final database lock and

unblinding, all unblinded data analyses will be handled by the unblinded team of statisticians and programmers. A strict firewall between the blinded and unblinded teams will be maintained.

For the 2 planned interim analyses, BARDA, site personnel, and subjects will be unblinded for all interim data at Day 43 and Day 163 only at the study group level (i.e., data will be provided with each group having a fake designation). For the final analysis, all parties will be unblinded to all data at the subject level at Day 142 for all Groups A-L and Day 262 for Groups E, F, K and L.

With the exception of the unblinded study staff who administer the IP and a separate unblinded monitor who will monitor IP accountability, all other study staff are to remain blinded until CSR database freeze and a formal unblinding is scheduled. The members of the IDMC will review subject level unblinded safety data on an ad hoc basis when the Study Pausing Rules (Section 11.1) are met. A separate unblinded team will handle any ad hoc production of unblinded tables, listings, and figures for ad hoc IDMC meetings. Blinded individuals will not be made aware of any unblinded data provided to the IDMC.

4.3 Analysis Sets

4.3.1 All Enrolled Population

The All Enrolled Population will consist of all subjects who signed the informed consent form (ICF).

4.3.2 Full-Analysis Set (FAS)

The FAS will consist of all subjects who are randomly assigned to receive IP. All analyses using the FAS will group subjects according to randomized treatment.

4.3.3 Safety Set

The safety set will consist of all subjects who are randomly assigned and receive at least 1 dose of IP. Safety endpoint summaries and listings will be performed on the safety set using study group treatment actually received.

4.3.4 Immunogenicity-Full Analysis Set (IFAS)

The IFAS will consist of all subjects who are randomly assigned, receive at least 1 dose of IP, and have at least 1 valid postvaccination and determinate assay result. As supportive analysis, all the immunogenicity endpoint summaries will be performed on the IFAS by actual treatments received.

4.3.5 Immunogenicity Per-Protocol Set (IPPS)

The IPPS is a subset of the IFAS, where all subjects meet the IFAS criteria and must meet the following criteria:

- have received a full dose of IP at all planned dosing visits.
- have received all the doses of IP to which they were randomly assigned.
- have no significant protocol deviations that are determined to potentially interfere with the immunogenicity assessment of IP.

- have received Visit 4 (Day 22) vaccination within -3 to +10 days of the expected visit date (21 days from Day 1) for all Groups, and Visit 8 (Day 142) vaccination within ±14 days of the expected visit date (141 days from Day 1) for Groups E, F, K and L.
- have Visit 7 (Day 43) immunogenicity sample collected within -3 to +10 days of the expected visit date (21 days f rom Visit 4 [Day 22]) for Groups A-D and G-J, and Visit 11 (Day 163) immunogenicity sample collected within -3 to +10 days of the expected visit date (21 days from Visit 8 [Day 142]) for Groups E, F, K and L.

All the immunogenicity endpoint summaries will be performed primarily on the IPPS using treatment actually received.

5 Subject Disposition

5.1 Disposition

The number and percentage of subjects who were randomized, completed Day 142 visit or did not complete Day 142 visit (including reason for not completing Day 142 visit) for groups A-D, G-J and completed Day 262 visit or did not complete Day 262 visit (including reason for not completing Day 262 visit) for groups E, F, K and L, and treated according to each dose will be summarized by randomized treatment and overall based on the FAS. A similar summary by site will also be provided.

A corresponding listing will be provided with subject-level data including randomization status, Day 142 visit completion/discontinuation status, date, and reason for not completing Day 142 visit (if applicable) and Day 262 visit completion/discontinuation status, date, and reason for not completing Day 262 visit (if applicable).

The disposition table for the addendum will include summary of number and percentage of subjects who completed or discontinued study along with primary reason for study discontinuation.

5.2 Protocol Deviations

Protocol deviations will be summarized by categories, randomized treatment and overall based on FAS. Additionally, the number and percentage of subjects with at least one protocol deviation and the number and percentage of subjects with at least one significant protocol deviation will be summarized. A significant deviation occurs when there is nonadherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject or risk to validity of study outcomes. Significant deviations can include nonadherence to inclusion or exclusion criteria or nonadherence to Food and Drug Administration (FDA) regulations or ICH GCP guidelines, and may lead to the subject being withdrawn from the study. Significant deviations will be identified based on blinded data reviews of the deviation log throughout the study and will be finalized prior to database lock. Significant deviations will be summarized by categories, randomized treatment and overall based on FAS. A corresponding listing will be presented.

6 Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized using the Safety Set, IPPS and IFAS by treatment received. In the event that a subject incorrectly receives doses of IP different from group A-L regimen, the subject will be included in the listings. A separate summary by site based on Safety Set will also be provided.

6.1 Demographics

Continuous Variables:

- Baseline age (years) from subject age entered directly in IVRS at Visit 1
- Height (in)
- Weight (lb)
- Body mass index (BMI) (kg/m²)

Specifications for computation:

- BMI (kg/m^2) = weight (lb)*703/[height (in)]^2

Categorical Variables:

- Baseline age (years) 18-<30, 30-<40, and 40-49
- Sex (Male, Female)
- Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Other, and Multi-racial)
- Ethnicity (Hispanic or Latino, and Not Hispanic or Latino)
- Subject travelled outside the United States after 2005 (Yes, No)
- Subject lived outside the United States for 3 months or more after 2005 (Yes, No)
- A corresponding listing will be provided with subject-level demographic data. The summary of vaccines received 12 months prior to Visit 1 will also be included in a data listing.

6.2 Alcohol, Drug and Tobacco Usage

The number and percentage of subjects with alcohol, drug or tobacco usage status of "current", "previous", or "never" will be summarized by treatment received based on the Safety Set. Within each type of tobacco, the number and percentage of subjects with usage status of "current", "previous", and "never" will be summarized. Type of tobacco includes cigarettes, cigars, pipes, and chewing tobacco.

Alcohol and tobacco usage for the Safety Set will also be included in a data listing containing all subject-level data collected in the eCRF.

6.3 Medical History

A summary for medical history will be presented by body system code and by treatment received based on the Safety Set. If a subject experiences multiple occurrences of medical history of the same body system code, it will be counted only once for that category. Additionally, the number and percentage of subjects with at least 1 medical history will be summarized.

Medical history for the Safety Set will be included in a data listing containing all subject-level data collected in the eCRF.

6.4 Inclusion and Exclusion Criteria

A data listing will be displayed for subjects who did not meet inclusion criteria or subjects who met exclusion criteria for the FAS. Only the inclusion criteria not met and the exclusion criteria met will be listed.

7 Treatments and Medications

7.1 Prior and Concomitant Medications

Use of all concomitant medications that the subject is taking at the time of Visit 1 (Day 1), medications taken up to 14 days prior to Visit 1 (Day 1), and vaccines received 12 months prior to Visit 1 (Day 1) will be collected in the eCRF. All medications will be coded according to the World Health Organization drug dictionary.

A prior medication is defined as any medication that is taken within 12 months prior to the first dose of IP. A concomitant medication is defined as any medication that has a stop date that is on or after the date of first dose of IP. A medication can be considered as both prior and concomitant medication when the start date is before the first dose of IP, but the stop date is on or after the first dose of IP.

Prior medications and concomitant medications will be summarized separately by presenting the number and percentage of subjects having any prior/concomitant medications and having each individual prior/concomitant medication based on Anatomical Therapeutic Chemical (ATC) level 4 and Preferred Term (PT). At each level of subject summarization, a subject is counted once if the subject reported one or more medications. Medications will be sorted in descending order by frequency of ATC level 4 category and preferred terms.

Prior and concomitant medications data collected will be presented in a data listing. A separate listing of concomitant medications collected after Day 142 for the 2-dose vaccination series and Day 262 for the 3-dose vaccination series will be included in the addendum to the CSR once the study is complete.

7.2 Exposure

The number and percentage of subjects who received 1 dose, 2 doses and 3 doses (Groups E, F, K and L only) of IP will be summarized, respectively, based on the Safety Set by treatment received. For the summary of 2 doses and 3 doses of IP, the summary numbers will be presented by doses as randomized and doses different from randomized if detected.

A corresponding listing will be provided with subject-level IP administration details. Also, a listing of subjects with vaccines different from randomized will be provided.

8 Immunogenicity Analysis

Immunogenicity analyses will be performed primarily on IPPS by actual treatment received. IFAS will be the supportive analysis set for immunogenicity analysis based on actual treatments. If HAI or MN titer is undetectable, it will be assigned a value of half the lower limit of detection.

SPR for HAI antibody responses is defined as proportion of subjects achieving a serum HAI titer of at least 1:40 against each vaccine strain in the study. Analysis of SPR for HAI antibody response will be conducted using both IFAS and IPPS.

SCR for HAI or MN antibody responses is defined as proportion of subjects achieving either a prevaccination HAI or MN titer of <1:10 and a postvaccination titer of at least 1:40 or a prevaccination HAI or MN titer of at least 1:10 and a 4-fold or greater increase of HAI or MN postvaccination antibody titers against each vaccine strain in the study. Analysis of SCR and GMTs for HAI or MN antibody responses will be conducted using IPPS only.

A corresponding listing will be provided with individual immunogenicity results per subject, antibody test (HAI or MN), tested strains, and time point based on IFAS.

8.1 Primary Analysis

The serum HAI antibody SPR and corresponding asymptotic 95% CIs against the strains contained in the last study vaccines on Day 43 of the prime-boost vaccination series (Groups A-D, G-J) and on Day 163 of the prime-prime-boost vaccination series (Groups E, F, K and L) will be summarized for each treatment group. Bar charts will be presented for SPR.

8.2 Secondary Analysis

- Geometric mean titers (GMTs) of HAI antibody responses against each strain vaccinated at Day 1, 22, 43, and 142 visits (Groups A-D, G-J) and Day 1, 22, 43, 142, 163 and 262 visits (Groups E, F, K and L) and asymptotic 95% CIs will be summarized along with other descriptive statistics, including number of subjects, geometric standard deviation, minimum, maximum, and median for each treatment group. Line plots will be provided for GMT of HAI and corresponding 95% CI for all visits.
- GMTs of MN antibody responses against each strain vaccinated at Day 1, 22, 43, and 142 visits (Groups A-D, G-J) or Day 1, 22, 43, 142, 163 and 262 visits (Groups E, F, K and L) and asymptotic 95% CIs will be summarized along with other descriptive statistics, including number of subjects, geometric standard deviation, minimum, maximum, and median for each treatment group. Line plots will be provided for GMT of MN and corresponding 95% CI for all visits.
- SPR for HAI antibody responses against each strain vaccinated at Day 1, 22, 43, and 142 visits (Groups A-D, G-J) or Day 1, 22, 43, 142, 163 and 262 visits (Groups E, F, K and L) along with asymptotic 95% CIs will be summarized. SPR for the combinations of HAI antibody responses against multiple strains vaccinated will be also summarized for each treatment group. Line plots will be provided for SPR for HAI and corresponding 95% CI for all visits.
- SCR for HAI antibody responses against each strain vaccinated at Day 22, 43, and 142 visits (Groups A-D, G-J) or Day 22, 43, 142, 163 and 262 visits (Groups E, F, K and L) with asymptotic 95% CIs will be summarized for each treatment group. Line plots will be provided for SCR for HAI and corresponding 95% CI for all visits.
- SCR for MN antibody responses against each strain vaccinated at Day 22, 43, and 142 visits (Groups A-D, G-J) or Day 22, 43, 142, 163 and 262 visits (Groups E, F, K and L) with asymptotic 95% CIs will be summarized for each treatment group. Line plots will be provided for SCR for MN and corresponding 95% CI for all visits.

8.3 Exploratory Analysis

Exploratory analyses will be performed to compare immunogenicity endpoints between specified study groups. All exploratory analyses will be performed by treatment received based on IPPS. The following group-wise (targeted group vs. reference group) comparisons will be performed for GMT and SPR of HAI antibody responses:

- For strain A/gyrfalcon/Washington/41088-6/2014 H5N8 (Seqirus) on Day 43:
  - Study Group A vs. Study Group E
  - Study Group B vs. Study Group E
  - Study Group C vs. Study Group E
  - Study Group D vs. Study Group E
  - Study Group F vs. Study Group E
  - Study Group G vs. Study Group K
  - Study Group H vs. Study Group K
  - Study Group I vs. Study Group K
  - Study Group J vs. Study Group K
  - Study Group L vs. Study Group K
- For strain A/barheaded goose/Qinghai Lake/1A/2005 (H5N1) (Sanofi Pasteur) on Day 163:
  - Study Group E vs. Study Group F
  - Study Group K vs. Study Group L

The logarithms of HAI antibody titers will be compared by two-sided Student's t-test between above specified study groups. Geometric mean ratio estimate will also be presented for each pair of group comparison with corresponding asymptotic 95% CIs and p-values based on two sample t-test.

The difference in proportions (SPR for targeted group-SPR for reference group) between the above specified study groups will be presented along with corresponding asymptotic 95% CIs and p-values based on Chi-square test.

Based on actual results, additional exploratory study group comparisons may be performed.

9 Safety Analysis

All safety evaluations will be performed using the Safety Set.

9.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to IP.

An SAE is defined as any event that results in death, is immediately life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the

subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Medically attended AEs are defined as AEs with medically attended visits, including hospital, emergency room, urgent care clinic, or other visits to or from medical personnel (medical doctor, physician assistant, nurse practitioner) for any reason.

Potentially immune-mediated medical conditions are considered adverse events of special interest. The PIMMCs of concern for this study are detailed in Appendix 2, Section 14.2.

AEs are categorized into 'Solicited Local AEs', 'Solicited Systemic AEs', and 'Unsolicited AEs' for AE type. Solicited Local AEs will include local reactions at the injection site with an onset within the 8 days after each vaccination, such as erythema/redness, induration/swelling, and pain. Solicited Systemic AEs will include systemic reactions with an onset within the 8 days after each vaccination, such as fever, myalgia, arthralgia, fatigue, headache, nausea, vomiting, diarrhea, and chills. All solicited local and systemic reactions will be considered as related to IP by the investigator. All other AEs which are not solicited are considered unsolicited.

Days of onset for unsolicited AEs are categorized into '1-8 Days' and '>8 Days' from each IP administration.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA)®, which provides the primary system organ class (SOC) and preferred term (PT) information.

All AEs reported after the first dose of IP will be summarized in tables. AEs within 21 days of each dose of IP, and before the date and time of the next dose of IP (if applicable) will be summarized in tables based on each dose only (Dose 1, 2 for Groups A-D, G-J; Dose 1, 2, 3 for Group E, F, K and L). When the time of an AE is missing and the AE start date is on the date of vaccination, then that AE will be considered as happening after the corresponding dose of IP.

All AEs will be summarized by presenting the number and percentage of subjects having any AEs and having each individual adverse event based on SOC and PT, and PT only. At each level of subject summarization, a subject is counted once if the subject reported one or more events. Percentages will be calculated out of the number of subjects as treated per study group in the Safety Set. For the dose-specific AE summaries, percentages will be calculated out of the number of subjects as treated per study group corresponding to Dose 1, Dose 2 or Dose 3.

Unless otherwise stated, AEs will be sorted in descending order by frequency of SOC and PT.

The following summaries, based on Dose 1 only, Dose 2 only, and both Dose 1 and Dose 2 for Groups A-D, G-J and Dose 1 only, Dose 2 only, Dose 3 only, and all Dose 1, Dose 2 and Dose 3 for Groups E, F, K and L will be provided:

- Overview summary of the number and percentage of subjects with any AE, SAE, AE related to IP, AE leading to study withdrawal, AE leading to death, solicited local AE, severe solicited local AE, solicited systemic AE, severe solicited systemic AE, MAAE, PIMMC, unsolicited AE, severe unsolicited AE, and unsolicited AE related to IP
- All AEs by SOC and PT
- All AEs by AE Type and PT
- All AEs by SOC, PT and Severity

- All AEs by SOC, PT and Relationship
- All unsolicited AEs by SOC, PT and Day of Onset

The following summaries with 95% exact CIs using Clopper Pearson method, based on events recorded during the 8 days post each vaccination will be provided as the analysis of primary safety endpoint:

- Solicited Local AEs by Severity
- Solicited Systemic AEs by Severity

All MAAEs and PIMMCs after the first dose of IP will be summarized in a table by SOC, PT and Severity.

In all by-severity summaries, the severity that will be presented represents the greatest severity captured in the eCRF if a subject reported multiple occurrences of the same AE. The possible severities include “Mild”, “Moderate” and “Severe”. The missing severity will be imputed as “Severe”.

In all by-relationship summaries, the relationship that will be presented represents the greatest relatedness captured in the eCRF if a subject reported multiple occurrences of the same AE. The possible relationship includes “Related” and “Unrelated”. The missing relationship will be imputed as “Related”.

All AEs will be included in a data listing containing all subject-level data collected in the eCRF. All SAEs, all AEs leading to study withdrawal, MAAEs, PIMMCs and severe AEs will be listed separately.

A listing of SAEs and MAAEs, including a subset of specific PIMMCs for 12 months after the last dose of study vaccine will be presented in the addendum to the CSR once the study is complete.

9.2 Vaccination Site Examinations

The vaccination site examinations include pain, erythema/redness, induration/swelling, and other local symptoms at the site of injection and will be conducted 30 minutes and 8 days post each vaccination, ET if clinically indicated, and unscheduled visits (if the unscheduled visit occurs within 8 days post each vaccinations). The size of measurable erythema/redness and induration/swelling will be categorized as: <2.5 cm, 2.5 – 5 cm, 5.1 – 10 cm, and >10 cm. Vaccination site examination results will be classified according to the FDA Toxicity Grading Scale (See Appendix 3, Section 14.3, Grade 1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Potentially Life Threatening) based on quantifiable criteria. The number and percentage of subjects who have vaccination site reactions involving pain, erythema/redness, induration/swelling, and other local symptoms will be summarized separately for each vaccination site examination period. In addition, the number and percentage of subjects who have vaccination site reactions involving measurable redness and swelling will be summarized by size category. This summary will be performed on the Safety Set by treatment received according to first, second or third dose as appropriate.

All vaccination site examination results will be included in a data listing containing all subject-level data collected in the eCRF. All subjects with severe reaction based on injection site examination will be included in a data listing.

9.3 Clinical Laboratory Evaluations

Laboratory assessments will be performed by a central laboratory. All summaries will be based on the units provided by the central laboratory, no conversion will be done. Clinical safety laboratory evaluations will include the following: hematology, coagulation and chemistry/metabolic panel. Clinical chemistry and hematology laboratory test results will be classified according to the FDA Toxicity Grading Scale (See Appendix 3, Section 14.3, Grade 1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Potentially Life Threatening) based on quantifiable criteria; lab results that are normal will be classified as Grade 0. This categorical data will be summarized in shift tables comparing by visit and by maximum post-baseline value with those at the baseline visit. Results will also be classified by the central laboratory as "Low", "Normal", or "High" based on normal reference ranges.

Summary tables presenting observed values and changes from baseline will be displayed for clinical laboratory tests with numeric values by study group for subjects in the Safety Set.

All laboratory results will be included in a data listing containing all subject-level data collected. Any laboratory finding that qualifies as AEs and nonqualifying laboratory abnormalities outside the laboratory's reference range will be toxicity graded according to the FDA Toxicity Grading Scale (Appendix 3, Section 14.3, Grade 1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Potentially Life Threatening). Subjects with any clinically significant lab abnormality will also be listed.

9.4 Vital Sign Measurements

Vital signs to be obtained include the following:

- Blood pressure (systolic and diastolic [mm/Hg])
- Pulse rate (beats per minute)
- Oral temperature (°F)

Vital signs results will be classified according to the FDA Toxicity Grading Scale (See Appendix 3, Section 14.3, Grade 1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Potentially Life Threatening) based on quantifiable criteria; vital signs results that are normal will be classified as Grade 0. This categorical data will be summarized in shift tables comparing by visit and by maximum post-baseline value with those at the baseline visit.

Summary tables presenting observed values and changes from baseline will be displayed for vital sign data by study group for subjects in the Safety Set.

All vital sign data by subject will be presented in a listing. Subjects with any clinically significant vital sign measurements will also be listed.

9.5 Physical Examination

An assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, musculoskeletal system/extremities, and neurological system will be done at all applicable visits. Any complete physical examination finding will be captured as medical history and any targeted physical examination will be collected for AEs. All physical examination results for the Safety Set will be presented in a listing.

9.6 Pregnancy

Urine pregnancy test results for the Safety Set will be included in a data listing containing all subject-level data collected in the eCRF.

10 Interim Analysis

Interim analyses will be performed based on cumulative immunogenicity and safety data at Day 43 and Day 163 for all study groups. At each time point the study database will be monitored, cleaned, and frozen. The study team, except the unblinded team members, should remain blind until final database lock and unblinding. Interim analysis results will be unblinded at the group level and provided to BARDA by the unblinded team. A selection of safety and immunogenicity analyses in section 5 to section 9 will be included in the interim analyses. Interim data will be presented in tables, listings and figures.

11 Ad Hoc Safety Analysis for Independent Data Monitoring Committee (IDMC)

11.1 Study Pausing Rules

Study pausing rules will be monitored throughout the study by the medical monitor. The sponsor will pause study enrollment and study vaccine administration and the IDMC will review unblinded safety data if any of the following are met:

- Occurrence of one or more SAE(s), MAAE(s), or PIMMC(s) judged to be related to IP; OR
- Any potentially life-threatening AE judged to be related to IP (An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death); OR
- A death, if judged to be related to IP; OR
- Anaphylaxis or bronchospasm within 4 hours of injection judged to be related to IP; OR
- 10% of vaccinated subjects at a given vaccination time point when at least 50% of subjects in that group have been vaccinated experience the same or similar systemic or local Grade 3 (toxicity scoring) or higher event; OR
- A pattern of significant symptoms, physical findings, or laboratory abnormalities that, although individually minor, collectively represent a safety concern in the opinion of the investigator or the medical monitor.

If any of the above rules is met, the study will be paused. An unblinded safety report will be submitted to IDMC for review. The safety report will include summary of study disposition and demographic information for all randomized subjects and adverse events for safety set. Also, listing of protocol deviations, all adverse events, vaccination site examination for subjects with severe reaction, significant physical findings, vital signs and laboratory results for subjects with toxicity grade 3 or above will be provided to IDMC for review.

12 References

1. Afluria, Influenza Virus Vaccine [package insert]. CSL Limited (Australia); 2010.
2. Fluzone Quadrivalent (Influenza Vaccine) [package insert]. Sanofi Pasteur Inc. Swiftwater (PA); 2016.
3. Flucelvax Quadrivalent (Influenza Vaccine) [package insert]. Seqirus, Inc. Holly Springs (NC); 2016.
4. Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted; Emulsion for Intramuscular Injection [package insert]. Quebec City (QC, Canada); GlaxoSmithKline 2013.
5. SAS Institute Inc. SAS® Procedures Guide, Version 9.3. Cary, NC: SAS Institute Inc. 2008.

13 Analyses Deviated From Protocol

Following exploratory objectives for immunogenicity described in protocol are not included in this SAP:

- To assess peripheral antibody responses to epitopes within the influenza virus surface proteins hemagglutinin or neuraminidase induced by prime-boost or prime-prime-boost vaccination series.
- To examine cell-mediated (e.g., B cell, CD4+ T cell, CD8+ T cell) responses in a subset composed of approximately half of enrolled subjects using peripheral blood mononuclear cells collected at Day 1, Day 22, Day 43, and Day 142 (Groups A-D, G-J) or Day 1, Day 22, Day 43, Day 142, Day 163, and Day 262 (Groups E, F, K and L).

14 Appendices

14.1 Appendix 1: Schedule of Events

Table 14-1 Schedule of Events, Groups A to D, G to J

Study visit	Screening	V1	V2[a]	V3	V4	V5[a]	V6	V7	V8	V9[a] (EOS)	ET	Unsch
Study day	**-14 to -3**	**1**	**4**	**8**	**22**	**25**	**29**	**43**	**142**	**387**	**N/A**	**N/A**
Window allowance (days)			**V1+3 (±1)**	**V1+7 (±1)**	**V1+21 (±3)**	**V4+3 (±1)**	**V4+7 (±1)**	**V4+21 (±3)**	**V1+141 (±7)**	**V1+386 (±7)**	**N/A**	**N/A**
Obtain informed consent	X[b]											
Vital sign measurements[c]	X	X		X	X		X	X			X	X[d]
Height and weight	X	X[e]										
Medical history	X	X[e]										
Perform subject interview[f]			X	X	X	X	X	X	X	X	X	X
Review diary card[g]			X	X	X	X	X	X			X[h]	X[h]
Distribute diary card[g] and review instructions		X DCI		X DCII	X DCIII		X DCIV					
Distribute measuring tool and thermometer and review instructions		X			X							
Collect diary card[g]				X DCI	X DCII		X DCIII	X DCIV				
Concomitant medications	X[i]	X[e,i]	X	X[j]	X[e]	X	X[j]	X	X	X	X	X
Concomitant vaccinations	X[i]	X[e,i]	X	X[j]	X[e]	X	X[j]	X	X	X	X	X
Complete physical examination[k]	X											
Targeted physical examination[l]		X[e]		X	X[e]		X				X[d]	X[d]
Urine pregnancy test	X	X[e,m]			X[e,m]							X[d]
Review inclusion and exclusion criteria	X	X[e]			X[e]							

Table 14-1 Schedule of Events, Groups A to D, G to J

Study visit	Screening	V1	V2[a]	V3	V4	V5[a]	V6	V7	V8	V9[a] (EOS)	ET	Unsch
Study day	**-14 to -3**	**1**	**4**	**8**	**22**	**25**	**29**	**43**	**142**	**387**	**N/A**	**N/A**
Window allowance (days)			**V1+3 (±1)**	**V1+7 (±1)**	**V1+21 (±3)**	**V4+3 (±1)**	**V4+7 (±1)**	**V4+21 (±3)**	**V1+141 (±7)**	**V1+386 (±7)**	**N/A**	**N/A**
Venous blood sample collection for clinical safety laboratory tests[n]	X			X[o]	X[e,o]		X[o]					X[d]
Venous blood sample collection for antibody assays		X[e]			X[e]			X	X			
Cellular immunology (subset only)		X[e]			X[e]			X	X			
Randomization		X[e]										
Vaccination		X			X[p]							
Monitor subject for 30 minutes following vaccination[q]		X			X							
Examine vaccination site[r]		X[s]		X	X[s]		X				X[d]	X[t]
AE/MAAE /PIMMC/SAE assessment[u]		X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE, adverse event; DC, diary card; eCRF, electronic case report form; EOS, end of study; ET, early termination; MAAE, medically attended adverse event; PIMMC, potentially immune-mediated medical condition; N/A, not applicable; SAE, serious adverse event; SUSAR, suspected unexpected serious adverse reaction; Unsch, unscheduled; V, visit; WOCBP, women of childbearing potential.

[a] Telephone call assessment.

[b] Prior to the completion of any study-related procedures.

[c] Vital sign measurements include oral temperature, pulse rate, and blood pressure. Vital signs will be measured before blood is collected, at both pre-vaccination and 30±5-minute postvaccination time points on a vaccination day. Diastolic and systolic blood pressure will be measured after the subject is seated for at least 5 minutes.

[d] If clinically indicated.

[e] Prior to vaccination.

[f] Ask subject a standard nonleading question to elicit any medically related changes in their well-being. Ask if they have been hospitalized, had any accidents, used any new medications, received any vaccinations, or changed concomitant medication regimens (both prescription and over-the-counter medications).

[g] Diary Card I (Days 1-8) and III (Days 22-29) will be used to collect unsolicited and solicited local and systemic reactions. Diary Card II (Days 9-21) and IV (Days30-43) will be used to collect unsolicited AEs.

[h] If applicable, according to the protocol period, collect and review DC with subject.

[i] Use of all concomitant medications that the subject is taking at the time of Visit 1, medications taken up to 14 days prior to Visit 1, and vaccines received 12 months prior to Visit 1 will be recorded in the subject's eCRF.

[j] Obtained by interview and by review of diary card completed by the subject.

[k] Any physical examination findings will be collected in the eCRF for medical history data presentation.

[l] Targeted physical examination will be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities. Any findings from this examination will be collected in the eCRF for AEs.

[m] A urine pregnancy test must be performed within 24 hours prior to vaccination and pregnancy test results must be final and negative prior to vaccination.

[n] Hematology: complete blood count with differential; platelet count; coagulation: partial thromboplastin time and prothrombin time (international normalized ratio), chemistry/metabolic panel: alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatinine, and blood urea nitrogen.

[o] For abnormal laboratory results that are clinically significant, repeat test until resolved or results become stable.

[p] Subjects with an acute illness, including body temperature greater than 100.4°F, immediately prior to each vaccination or, per subject report, within 3 days prior to a scheduled vaccination in this study can be rescheduled for vaccination as long as the vaccination visit is within the visit window. Subjects with a Grade ≥3 AE that has not returned to baseline or stabilized will not be revaccinated.

[q] At Visits 1 and 4, subjects will be monitored for solicited and unsolicited AEs, SAEs, MAAEs, PIMMCs, and SUSARs for at least 30 minutes after vaccination; concomitant medications will be recorded.

[r] Examine vaccination site for erythema/redness and swelling and monitor for pain.

[s] At Visits 1 and 4, complete a 30±5-minute postvaccination injection site examination for erythema/redness and swelling and monitor for pain. If a visible injection-site abnormality is observed, document with a photograph.

[t] If the unscheduled visit occurs within 7 days after either of the 2 study vaccinations.

[u] Only MAAEs, PIMMCs (see Appendix 2, Section 14.2), and SAEs will be collected after Day 43, all other AEs are collected through 21 days following each vaccination.

Table 14-2 Schedule of Events, Groups E, F, K and L

Study visit	Screening	V1	V2[a]	V3	V4	V5[a]	V6	V7	V8	V9[a]	V10	V11	V12	V13[a]	V14[a] (EOS)	ET	Unsch
Study day	**-14 to -3**	**1**	**4**	**8**	**22**	**25**	**29**	**43**	**142**	**145**	**149**	**163**	**262**	**322**	**507**	**N/A**	**N/A**
Window allowance (days)			**V1+3 (±1)**	**V1+7 (±1)**	**V1+21 (±3)**	**V4+3 (±1)**	**V4+7 (±1)**	**V4+21 (±3)**	**V1+141 (±7)**	**V8+3 (±1)**	**V8+7 (±1)**	**V8+21 (±3)**	**V1+261 (±7)**	**V1+321 (±7)**	**V1+506 (±7)**	**N/A**	**N/A**
Obtain informed consent	X[b]																
Vital sign measurements[c]	X	X		X	X		X	X	X		X	X	X			X	X[d]
Height and weight	X	X[e]															
Medical history	X	X[e]															
Perform subject interview[f]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review diary card[g]			X	X	X	X	X	X		X	X	X				X[h]	X[h]
Distribute diary card[g] and review instructions		X DCI		X DCII	X DCIII		X DCIV		X DCV		X DCVI						
Distribute measuring tool and thermometer and review instructions		X			X				X								
Collect diary card[g]				X DCI	X DCII		X DCIII	X DCIV			X DCV	X DCVI					
Concomitant medications	X[i]	X[e,i]	X	X[j]	X[e]	X	X[j]	X	X[e]	X	X[j]	X	X	X	X	X	X

Table 14-2 Schedule of Events, Groups E, F, K and L

Study visit	Screening	V1	V2[a]	V3	V4	V5[a]	V6	V7	V8	V9[a]	V10	V11	V12	V13[a]	V14[a] (EOS)	ET	Unsch
Study day	**-14 to -3**	**1**	**4**	**8**	**22**	**25**	**29**	**43**	**142**	**145**	**149**	**163**	**262**	**322**	**507**	**N/A**	**N/A**
Window allowance (days)			**V1+3 (±1)**	**V1+7 (±1)**	**V1+21 (±3)**	**V4+3 (±1)**	**V4+7 (±1)**	**V4+21 (±3)**	**V1+141 (±7)**	**V8+3 (±1)**	**V8+7 (±1)**	**V8+21 (±3)**	**V1+261 (±7)**	**V1+321 (±7)**	**V1+506 (±7)**	**N/A**	**N/A**
Concomitant vaccinations	X[i]	X[e,i]	X	X[j]	X[e]	X	X[j]	X	X[e]	X	X[j]	X	X	X	X	X	X
Complete physical examination[k]	X																
Targeted physical examination[l]		X[e]		X	X[e]		X	X	X[e]		X					X[d]	X[d]
Urine pregnancy test (WOCBP)	X	X[e,m]			X[e,m]				X[e,m]								X[d]
Review inclusion and exclusion criteria	X	X[e]			X[e]				X[e]								
Venous blood sample collection for clinical safety laboratory tests[n,o]	X			X	X		X		X		X						X[d]

Table 14-2 Schedule of Events, Groups E, F, K and L

Study visit	Screening	V1	V2[a]	V3	V4	V5[a]	V6	V7	V8	V9[a]	V10	V11	V12	V13[a]	V14[a] (EOS)	ET	Unsch
Study day	**-14 to -3**	**1**	**4**	**8**	**22**	**25**	**29**	**43**	**142**	**145**	**149**	**163**	**262**	**322**	**507**	**N/A**	**N/A**
Window allowance (days)			**V1+3 (±1)**	**V1+7 (±1)**	**V1+21 (±3)**	**V4+3 (±1)**	**V4+7 (±1)**	**V4+21 (±3)**	**V1+141 (±7)**	**V8+3 (±1)**	**V8+7 (±1)**	**V8+21 (±3)**	**V1+261 (±7)**	**V1+321 (±7)**	**V1+506 (±7)**	**N/A**	**N/A**
Venous blood sample collection for antibody assays		X[e]			X[e]			X	X[e]			X	X				
Cellular immunology (subset only)		X[e]			X[e]			X	X[e]			X	X				
Randomization		X[e]															
Vaccination		X			X[p]				X								
Monitor subject for 30 minutes following vaccination[q]		X			X				X								
Examine vaccination site[r]		X[s]		X	X[s]		X[d]		X[s]		X					X[d]	X[t]
AE/MAAE/ PIMMC/SAE assessment[u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE, adverse event; DC, diary card; eCRF, electronic case report form; EOS, end of study; ET, early termination; MAAE, medically attended adverse event; PIMMC, potentially immune-mediated medical condition; N/A, not applicable; SAE, serious adverse event; SUSAR, suspected unexpected serious adverse reaction; Unsch, unscheduled; V, visit; WOCBP, women of childbearing potential.

[a] Telephone call assessment.

[b] Prior to the completion of any study-related procedures.

[c] Vital sign measurements include oral temperature, pulse rate, and blood pressure. Vital signs will be measured before blood is collected, at both pre-vaccination and 30±5-minute postvaccination time points on a vaccination day. Diastolic and systolic blood pressure will be measured after the subject is seated for at least 5 minutes.

[d] If clinically indicated.

[e] Prior to vaccination.

[f] Ask subject a standard nonleading question to elicit any medically related changes in their well-being. Ask if they have been hospitalized, had any accidents, used any new medications, received any vaccinations, or changed concomitant medication regimens (both prescription and over-the-counter medications).

[g] Diary Card I (Days 1-8), III (Days 22-29), and V (Days 142-149) will be used to collect unsolicited and solicited local and systemic reactions. Diary Card II (Days 9-21), IV (Days 30-43), VI (Days 150-163) will be used to collect unsolicited AEs.

[h] If applicable, according to the protocol period, collect and review DC with subject.

[i] Use of all concomitant medications that the subject is taking at the time of Visit 1, medications taken up to 14 days prior to Visit 1, and vaccines received 12 months prior to Visit 1 will be recorded in the subject's eCRF.

[j] Obtained by interview and by review of diary card completed by the subject.

[k] Any physical examination findings will be collected in the eCRF for medical history data presentation.

[l] Targeted physical examination will be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities. Any findings from this examination will be collected in the eCRF for AEs.

[m] A urine pregnancy test must be performed within 24 hours prior to vaccination and pregnancy test results must be final and negative prior to vaccination.

[n] Hematology: complete blood count with differential; platelet count; coagulation: partial thromboplastin time and prothrombin time (international normalized ratio), chemistry/metabolic panel: alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatinine, and blood urea nitrogen.

[o] For abnormal laboratory results that are clinically significant, repeat test until resolved or results become stable.

[p] Subjects with an acute illness, including body temperature greater than 100.4°F, immediately prior to each vaccination or, per subject report, within 3 days prior to a scheduled vaccination in this study can be rescheduled for vaccination as long as the vaccination visit is within the visit window. Subjects with a Grade ≥3 AE that has not returned to baseline or stabilized will not be revaccinated.

[q] At Visits 1, 4, and 8, subjects will be monitored for solicited and unsolicited AEs, SAEs, MAAE, PIMMCs, and SUSARs for at least 30 minutes after vaccination; concomitant medications will be recorded.

[r] Examine vaccination site for erythema/redness and swelling and monitor for pain.

[s] At Visits 1, 4, and 8, complete a 30±5-minute postvaccination injection site examination for erythema/redness and swelling and monitor for pain. If a visible injection-site abnormality is observed, document with a photograph.

[t] If the unscheduled visit occurs within 7 days after any of the study vaccinations.

[u] Only MAAEs, PIMMCs (see Appendix 2, Section 14.2), and SAEs will be collected after Day 163; all other AEs will be collected through 21 days following each vaccination.

14.2 Appendix 2: Potentially Immune-Mediated Medical Conditions (PIMMC)

An adverse event of special interest (serious or nonserious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (e.g., regulators) might also be warranted (based on Council for International Organizations of Medical Sciences VI).

For this study, adverse events of special interest will include the following list of PIMMCs:

Gastrointestinal disorders

- Autoimmune pancreatitis
- Celiac disease
- Crohn's disease
- Microscopic colitis
- Ulcerative colitis
- Ulcerative proctitis

Liver disorders

- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Metabolic diseases

- Addison's disease
- Autoimmune hypophysitis
- 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., noninfectious encephalitis, encephalomyelitis, myelitis, radiculomyelitis)
- 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 the following: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and antineutrophil 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 neutropenia
- Autoimmune pancytopenia
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Polyglandular autoimmune syndrome
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis

14.3 Appendix 3: US FDA Guidance for Industry: FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (a Partial List)

http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalization
Erythema/redness[a]	2.5-5 cm	5.1-10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/swelling[b]	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[b] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C)[b] (°F)[b]	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	39.0-40 102.1-104	>40 >104
Tachycardia – beats per minute	101-115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute[c]	50-54	45-49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141-150	151-155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91-95	96-100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85-89	80-84	<80	ER visit or hospitalization for hypotensive shock
Respiratory rate – breaths per minute	17-20	21-25	>25	Intubation

Abbreviation: ER, emergency room.

[a] Subject should be at rest for all vital sign measurements.

[b] Oral temperature; no recent hot or cold beverages or smoking.

[c] When resting heart rate is between 60 to 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 g/24 hours	4-5 loose stools or 400-800 g/24 hours	6 or more watery stools or 800 g/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non--narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with daily activity	Some interference with daily activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Abbreviations: ER, emergency room; IV intravenous.

Serum[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Blood urea nitrogen mg/dL	23-26	27-31	>31	Requires dialysis
Creatinine mg/dL	1.5-1.7	1.8-2.0	2.1-2.5	>2.5 or requires dialysis
Liver function tests – ALT, AST increase by factor	1.1-2.5 × ULN	2.6-5.0 × ULN	5.1-10 × ULN	>10 × ULN
Bilirubin – when accompanied by any increase in liver function test increase by factor	1.1-1.25 × ULN	1.26-1.5 × ULN	1.51-1.75 × ULN	>1.75 × ULN
Bilirubin – when liver function test is normal; increase by factor	1.1-1.5 × ULN	1.6-2.0 × ULN	2.0-3.0 × ULN	>3.0 × ULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[b] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4).

Hematology[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (female) – g/dL	11.0-12.0	9.5-10.9	8.0-9.4	<8.0
Hemoglobin (female) change from baseline value – g/dL	Any decrease – 1.5	1.6-2.0	2.1-5.0	>5.0
Hemoglobin (male) – g/dL	12.5-13.5	10.5-12.4	8.5-10.4	<8.5
Hemoglobin (male) change from baseline value – g/dL	Any decrease – 1.5	1.6-2.0	2.1-5.0	>5.0
WBC increase – cell/mm^3	10 800-15 000	15 001-20 000	20 001-25 000	>25 000
WBC decrease – cell/mm^3	2 500-3 500	1 500-2 499	1 000-1 499	<1 000
Lymphocytes decrease – cell/mm^3	750-1 000	500-749	250-499	<250
Neutrophils decrease – cell/mm^3	1 500-2 000	1 000-1 499	500-999	<500
Eosinophils – cell/mm^3	650-1 500	1501-5 000	>5 000	Hypereosinophilic
Platelets decrease – cell/mm^3	125 000-140 000	100 000-124 000	25 000-99 000	<25 000
PT – increase by factor	1.0-1.10 × ULN[b]	1.11-1.20 × ULN	1.21-1.25 × ULN	>1.25 ULN
PTT – increase by factor	1.0-1.2 × ULN	1.21-1.4 × ULN	1.41-1.5 × ULN	>1.5 ULN

Abbreviations: PPT, partial thromboplastin time; PT, prothrombin time; ULN, upper limit of normal; WBC, white blood cell.

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[b] ULN is the upper limit of the normal range.