

CLINICAL STUDY PROTOCOL V89_18 Version 3.0

A Phase 3 Randomized, Observer-Blind, Multi-center, Controlled Study to
Evaluate Safety, Immunogenicity, and Lot-to-Lot Consistency of an
Adjuvanted Cell Culture-Derived, H5N1 Subunit Influenza Virus Vaccine
in Healthy Adult Subjects ≥ 18 years of Age

BB-IND No 13,536

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PROTOCOL SYNOPSIS V89_18 VERSION 3.0

Name of Sponsor: Seqirus, Inc.	Protocol number: V89_18	BB-IND No. 13,536
Title of Study: A Phase 3 Randomized, Observer-Blind, Multi-center, Controlled Study to Evaluate Safety, Immunogenicity, and Lot-to-Lot Consistency of an Adjuvanted Cell Culture-Derived, H5N1 Subunit Influenza Virus Vaccine in Healthy Adult Subjects ≥ 18 years of Age		
Study Period: The study period for each subject will be approximately 13 months.		Clinical Phase: Phase 3
<p>Rationale: H5N1 type influenza virus is a specific strain that resulted in rapid spread of influenza in birds and poultry (bird flu) across Asia, Europe and Africa. This strain has been associated with 637 human cases documented from 2003 through 2013, with a case fatality rate of 59% (WHO 2013). Widespread circulation, pathogenicity and direct transmission of avian viruses to humans suggest that H5N1 has important pandemic potential, increased by the high case fatality rate. The Sponsor is developing an adjuvanted monovalent inactivated subunit vaccine against the H5N1 strain, as the availability of a vaccine (even if incompletely matched to the pandemic virus) in the early stages of a pandemic may prevent more infections and deaths than waiting for completely matched pandemic vaccines to be produced and distributed. The production of the viral antigen components is based on cell culture technology. This production process has been used for the production of Optaflu[®] (trivalent seasonal influenza vaccine, licensed in EU, and in the US under the trade name Flucelvax[™]) and Celtura[®] (monovalent, adjuvanted, pandemic H1N1 vaccine, licensed in Germany and other countries).</p> <p>Proprietary MF59[®] squalene-based oil-in-water emulsion adjuvant has been studied in a number of influenza vaccine formulations, and is included in a trivalent seasonal vaccine licensed for elderly adults in Europe, Flud[®], and in the registered cell culture-derived 2009 H1N1 pandemic vaccine, Celtura[®]. MF59 enhances the immune response in these seasonal and pandemic influenza vaccines and has a well-established safety profile . More than 100 million doses of MF59-adjuvanted influenza vaccines have been distributed in licensed products.</p> <p>The MF59-adjuvanted cell culture derived H5N1 vaccine (hereafter referred to as aH5N1c) has been tested in three Phase 2 studies (V89_04, V89_13 and V89_11) in adult, elderly adult, and pediatric subjects, respectively. These studies evaluated aH5N1c in two dosage levels, 7.5 mcg + 0.25 mL MF59 (full dose) and 3.75 mcg +</p>		

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<p>0.125 mL MF59 (half dose), in order to determine the lowest effective dose for each target population. Results from these phase 2 studies have led to the selection of the higher dose for the Phase 3 study in a healthy adult and elderly adult population.</p> <p>The purpose of this Phase 3 study is to evaluate the safety, immunogenicity and lot-to-lot consistency of 3 lots of aH5N1c vaccine in approximately 2394 healthy adults ≥ 18 years of age receiving the vaccine. Subjects will be randomized in a 3:1 ratio to receive either aH5N1c vaccine or saline placebo. Enrollment will be stratified by age: 18 to <65 years of age and ≥ 65 years of age, to allow adequate safety assessment of the entire age spectrum. In addition, both age cohorts will be evaluated for immunogenicity according to the Center for Biologics Evaluation and Research (CBER) and the Committee for Medicinal Products for Human Use (CHMP) criteria (CBER 2007, CHMP 2003, CHMP 2006).</p>		
<p>Primary Objectives</p> <p>Co-Primary Immunogenicity Objectives:</p> <p>To demonstrate lot-to-lot consistency across three consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of geometric mean titers (GMTs) of hemagglutination inhibition (HI) antibody responses to the H5N1 vaccine strain 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age.</p> <p>After lot-to-lot consistency is demonstrated, the populations of all H5N1 c vaccine recipients will be pooled in order to evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER guidance 3 weeks after the second vaccine administration (Day 43) as measured by age cohort and by strain-specific HI assay.</p> <p>Primary Safety Objective: To evaluate the safety and tolerability of aH5N1c vaccine and placebo in healthy adult subjects ≥ 18 years of age.</p> <p>Secondary Objectives</p> <p>Secondary Immunogenicity Objectives: If lot-to-lot consistency is demonstrated, the populations of vaccine recipients administered aH5N1c lots will be pooled and the following objectives assessed:</p> <ul style="list-style-type: none">• To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CHMP recommendations 3 weeks after the second vaccine		

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<p>administration (Day 43) in healthy adult subjects ≥ 18 years of age by age cohort¹, as measured by strain-specific HI assay.</p> <ul style="list-style-type: none"> To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER and CHMP recommendations 3 weeks after the first vaccine administration (Day 22) in healthy adult subjects ≥ 18 years of age by age cohort¹, as measured by strain-specific HI assay. To evaluate immune responses to aH5N1c vaccine 6 months after the first vaccine administration (Day 183) in healthy adult subjects ages ≥ 18 years of age, by age cohort, as measured by strain-specific HI assay. 																													
<p>Methodology: This is a Phase 3 stratified, randomized, observer-blind, multi-center, placebo-controlled study to evaluate safety, lot-to-lot consistency, and immunogenicity of aH5N1c in healthy adult subjects ≥ 18 years of age. Table 1 below outlines the study design.</p>																													
<p>Table 1: Study Design</p> <table border="1"> <thead> <tr> <th rowspan="2">Treatment Arm</th> <th rowspan="2">Schedule of Vaccine Administration</th> <th rowspan="2">Total Enrolled</th> <th colspan="2">Enrolled by Age Group</th> </tr> <tr> <th>Age 18 to <65 yrs</th> <th>Age ≥ 65 yrs</th> </tr> </thead> <tbody> <tr> <td>Group A: aH5N1c lot #1</td> <td>Day 1, Day 22</td> <td>798</td> <td>399</td> <td>399</td> </tr> <tr> <td>Group B: aH5N1c lot #2</td> <td>Day 1, Day 22</td> <td>798</td> <td>399</td> <td>399</td> </tr> <tr> <td>Group C: aH5N1c lot #3</td> <td>Day 1, Day 22</td> <td>798</td> <td>399</td> <td>399</td> </tr> <tr> <td>Group D: placebo</td> <td>Day 1, Day 22</td> <td>798</td> <td>399</td> <td>399</td> </tr> </tbody> </table>			Treatment Arm	Schedule of Vaccine Administration	Total Enrolled	Enrolled by Age Group		Age 18 to <65 yrs	Age ≥ 65 yrs	Group A: aH5N1c lot #1	Day 1, Day 22	798	399	399	Group B: aH5N1c lot #2	Day 1, Day 22	798	399	399	Group C: aH5N1c lot #3	Day 1, Day 22	798	399	399	Group D: placebo	Day 1, Day 22	798	399	399
Treatment Arm	Schedule of Vaccine Administration	Total Enrolled				Enrolled by Age Group																							
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Group D: placebo	Day 1, Day 22	798	399	399																									
<p>Enrolled subjects will be age-stratified to include approximately 1596 adults 18 to <65 years of age and approximately 1596 adults ≥ 65 years of age. Subjects will be randomized in a 1:1:1:1 ratio to receive 2 doses (on Day 1 and Day 22) of either one of the three consecutively produced aH5N1c vaccine lots or placebo (Groups A, B, C, and D) according to Table 1. Approximately 1197 subjects in each age stratum will receive</p>																													

¹ Both CBER and CHMP criteria will be evaluated separately for each age cohort (18 to <65 years and ≥ 65 years, and 18 to <60 years and ≥ 60 years respectively).

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<p>aH5N1c while 399 subjects will receive placebo. With 2394 subjects receiving active vaccine and allowing for 10% dropout, there will be approximately 2154 subjects evaluable for the primary immunogenicity objective.</p> <p>Vaccine will be administered in an observer-blind manner. After each vaccination, subjects will remain under medical supervision at the study site for at least 30 minutes for observation of immediate adverse events (AEs). After the second vaccine administration, subjects will be monitored for approximately 6 months for antibody persistence, and approximately 12 months for safety, for a total study duration of approximately 13 months per subject.</p> <p>The study is comprised of 17 scheduled contacts with each subject: 5 clinic visits, 4 reminder calls and 8 safety calls through a Treatment Period and a Follow-up Period:</p> <ul style="list-style-type: none">• Treatment Period: Day 1 through Day 42, including 2 clinic visits and 4 reminder calls.• Follow-up Period: Day 43 through Day 387, including 3 clinic visits and 8 safety calls. <p>Blood will be drawn from each subject for immunogenicity assessments before each vaccination (Day 1 and Day 22) and also on Day 43 and Day 183. Sera will be evaluated for antibody responses as measured by HI antibody responses against the H5N1 vaccine strain. Additional serologic testing methods (e.g., microneutralization (MN) testing) may be used as appropriate.</p> <p>Lot-to-lot consistency will be analyzed based on HI immune responses in approximately 798 subjects per aH5N1c lot. All subjects will be evaluated for safety, and immunogenicity against CBER and CHMP criteria.</p> <p>The subject diary cards will collect solicited AEs, unsolicited AEs, and medication/ vaccinations given from Day 1 to Day 7 (inclusive) and from Day 22 to Day 28 (inclusive). Unsolicited AEs, and solicited AEs that continue beyond Day 7 and 28 respectively, and medications/ vaccinations given to treat them, will be collected in the diary card until the time of return to the clinic on Day 22 and 43, respectively. In addition to these safety data, all serious adverse events (SAEs), all adverse events of special interest (AESI), new onset of chronic disease (NOCD), AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events, and all vaccinations will be collected from Day 1 through Day</p>		

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<p>387, inclusively. These data will be captured through the subject diary card, by interview of the subject, and by review of available medical records.</p> <p>Solicited local AEs will include: injection site induration, erythema, ecchymosis and pain. Solicited systemic AEs will include: nausea, generalized myalgia, generalized arthralgia, headache, fatigue, chills, loss of appetite, malaise and fever (derived from measured body [preferably oral] temperature ≥ 38.0 °C [≥ 100.4 °F]). Other AEs that will be measured will include body temperature and use of analgesic/antipyretic medication before and after vaccination.</p> <p>A detailed schedule and listing of procedures is shown in Table 2, Time and Events Table, included below.</p>		
Number of subjects planned: Approximately 3192 healthy adults ages 18 years and older, stratified into 1596 subjects aged 18 to <65 years, and 1596 subjects aged ≥ 65 years.		
Subject Population: Healthy male and females adult subjects ≥ 18 years of age, stratified in two equal age cohorts of 18 to <65 years of age and ≥ 65 years of age.		
Subject Characteristics and Key Criteria for Inclusion and Exclusion: Subjects ≥ 18 years of age, mentally competent, in good health as determined by medical history, physical examination and clinical judgment by the Investigator; able to comply with all study procedures, to be contacted, and to be available for study visits according to the protocol. Key Exclusion Criteria are: <ul style="list-style-type: none">- Individuals who are pregnant or breastfeeding. Female subjects of childbearing potential must have a negative pregnancy test prior to study vaccines being		

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<p>administered.</p> <ul style="list-style-type: none">- Females of childbearing potential² who refuse to use an acceptable method of birth control³ from Day 1 (1st vaccination) to 3 weeks after the second study vaccination, and, if sexually active, who have not used a reliable birth control method for at least two months prior to study entry.- Individuals with a body temperature ≥ 38.0 °C (≥ 100.4 °F) or any acute illness within 3 days of intended study vaccination.- Individuals who received any type of influenza vaccine (e.g., “seasonal”) within 7 days prior to enrolment in this study or who are planning to receive any type of influenza vaccine within 7 days (before or after) from the study vaccines.- Individuals who received any other licensed vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this study or who are planning to receive any (non-influenza) vaccine within 28 days (before or after) from the study vaccines.- Individuals with known or suspected impairment of the immune system. <p>Detailed lists of Inclusion and Exclusion Criteria are presented in Sections 4.1 and 4.2, respectively.</p> <p>Vaccines:</p> <p><u>Investigational vaccine:</u> MF59-adjuvanted cell-culture derived subunit inactivated monovalent A/H5N1 vaccine (aH5N1c) in Pre-Filled Syringes ready for use for IM administration, containing 7.5 mcg H5N1 hemagglutinin antigen (HA) + 0.25 mL MF59 (approximately 0.5 mL total volume).</p> <p><u>Control:</u> placebo (saline solution).</p>		

² Female of childbearing potential is defined as a post-onset menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, or (3) bilateral oophorectomy or hysterectomy.

³ Reliable birth control method is defined as sexual abstinence, hormonal (e.g., oral, injection, transdermal patch, implant, cervical ring), barrier (e.g., condom with spermicide or diaphragm with spermicide), intrauterine device (e.g., IUD), or monogamous relationship with partner who has been vasectomized for 6 months or more prior to the subject’s study entry.

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Immunogenicity Endpoints: The immunogenicity endpoints will be based on hemagglutinin inhibition (HI) antibody responses to the H5N1 vaccine strain. <ul style="list-style-type: none">- GMT at Day 43 by lot- GMT at Day 1, Day 22, Day 43 and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age; 18 to <60 years of age and ≥60 years of age)- Percentage of subjects with HI titer ≥ 1:40 at Day 1, Day 22, Day 43 and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age; 18 to <60 years of age and ≥60 years of age)- Percentage of subjects achieving seroconversion (defined as: HI titer ≥1:40 for subjects negative at baseline [HI titer <1:10]; or a minimum 4-fold increase in HI titer for subjects positive at baseline [HI titer ≥1:10]) at Day 22 and Day 43 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age; 18 to <60 years of age and ≥60 years of age)- Geometric mean ratio (GMR) at Day 1, Day 22, Day 43 and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥65 years of age; 18 to <60 years of age and ≥60 years of age)		
Safety Endpoints: The endpoints for assessing safety and tolerability are as follows: <ul style="list-style-type: none">- Percentages of subjects with solicited local, solicited systemic, and other adverse events as measured for 7 days following each vaccination within each vaccine group.- Percentages of subjects with unsolicited AEs reported through 21 days after last vaccination within each vaccine group.- Percentages of subjects reporting SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, and concomitant medications associated with these events as collected from Day 1 to Day 387 within each vaccine group.		

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Statistical Analysis of Primary Objectives: Co-Primary Immunogenicity Objectives: The lot-to-lot consistency will be claimed if the two-sided 95% confidence intervals (CIs) of all the three pairwise comparisons (GMT(Group A)/ GMT(Group B), GMT(Group A)/ GMT(Group C), GMT(Group C)/ GMT(Group B)) fall within 0.667 and 1.5. Significance level to all these tests is $\alpha = 5\%$, which needs no adjustment for multiplicity as all hypotheses have to be rejected (intersection-union test problem). With the proposed sample size, assuming a standard deviation of 0.85 for the log ₁₀ antibody titers (for each vaccine lot), approximate pairwise equivalence of factor 1 and independency, a single equivalence test based on 718 subjects per lot group has a power of 95%. The resulting overall power is approximately 86%, because the total number of comparisons is three. To account for dropouts (approximately 10%), a total of n=798 per lot should be recruited. After demonstrating success of the lot-to-lot consistency, the achievement of the CBER criteria (co-primary objective) will be claimed if the lower bound of the two-sided 95% CI for the percent of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 70% or 60% (for subjects 18 - <65 years of age and subjects ≥ 65 years of age, respectively) for all lots pooled. With the proposed sample size of 1077 per age cohort and the assumed proportions based on previous studies, such as V89_04 and V89_13 the power to meet or exceed 70% or 60% of the subjects achieving an HI antibody titer $\geq 1:40$ is 82% - > 99% (based on the age cohort). Primary Safety Objective: There is no statistical null hypothesis associated with the safety objective, which will be analyzed descriptively.		
Planned Analyses: An interim analysis of immunogenicity will be performed for all subjects through the Day 43 visit. Site staff and Sponsor personnel involved in study conduct will remain blinded to treatment assignments for this interim analysis. The final analysis will be performed when all data up to the study end (Day 387) are available. Analysis will be done on fully unblinded data set by the Sponsor and all data will be presented in the final study report.		
Data Monitoring Committee: No Data Monitoring Committee (DMC) is planned in		

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this study.		

Table 2: Times and Events Table, Scheduled Subject Contacts 1 – 17

Study Periods	Treatment Period						Follow-up Period										
Visit Number (V); Reminder Call (RC) Safety Call (SC); Study Day (D)	V1 D1	RC D3	RC D5	V2 D22	RC D24	RC D26	V3 D43	V4 SC D91	V5 SC D122	V6 SC D152	V7 D183	V8 SC D217	V9 SC D251	V10 SC D285	V11 SC D319	V12 SC D353	V13 D387
Visit Window	Day -5 to Day 1	2-3 days post 1st vac	4-5 days post 1st vac	21-30 days post 1st vac	2-3 days post 2nd vac	4-5 days post 2nd vac	21-30 days post 2nd vac	90 – 100 days post 1st vac	121-131 days post 1st vac	151-161 days post 1st vac	182 – 192 days post 1st vac	216- 225 days post 1st vac	250- 259 days post 1st vac	284-293days post 1st vac	318-327 days post 1st vac	352-361 days post 1st vac	386-395 days post 1st vac
Informed Consent ^a	X																
Medical history ^b	X																
Review of systems ^b	X			X			X				X						X
General physical examination ^b	X																
Symptom-directed physical examination ^b				X			X				X						X
Pregnancy test ^c (Women of childbearing potential)	X			X			X										
Measurement of body temperature (preferably oral) ^d	X			X													
Exclusion/Inclusion criteria ^e	X																
Enrollment and randomization ^f	X																

Study Periods	Treatment Period						Follow-up Period										
	V1 D1	RC D3	RC D5	V2 D22	RC D24	RC D26	V3 D43	V4 SC D91	V5 SC D122	V6 SC D152	V7 D183	V8 SC D217	V9 SC D251	V10 SC D285	V11 SC D319	V12 SC D353	V13 D387
Visit Window	Day -5 to Day 1	2-3 days post 1st vac	4-5 days post 1st vac	21-30 days post 1st vac	2-3 days post 2nd vac	4-5 days post 2nd vac	21-30 days post 2nd vac	90 – 100 days post 1st vac	121-131 days post 1st vac	151-161 days post 1st vac	182 – 192 days post 1st vac	216- 225 days post 1st vac	250- 259 days post 1st vac	284-293days post 1st vac	318-327 days post 1st vac	352-361 days post 1st vac	386-395 days post 1st vac
Serology blood draw (max: 12 mL whole blood) ^g	X			X			X				X						
Review criteria for repeat vaccination ^h				X													
Study vaccine administered ⁱ	X			X													
30 minute post-injection adverse event assessment ^j	X			X													
Diary card dispensed (and training on completion) ^j	X			X													
Diary card reminder call ^k		X	X		X	X											
Diary card reviewed and collected ^l				X			X										
Assess all AEs ^m	X			X			X										
Assess local and systemic solicited AEs ^l				X			X										

Study Periods	Treatment Period						Follow-up Period										
Visit Number (V); Reminder Call (RC) Safety Call (SC); Study Day (D)	V1 D1	RC D3	RC D5	V2 D22	RC D24	RC D26	V3 D43	V4 SC D91	V5 SC D122	V6 SC D152	V7 D183	V8 SC D217	V9 SC D251	V10 SC D285	V11 SC D319	V12 SC D353	V13 D387
Visit Window	Day -5 to Day 1	2-3 days post 1st vac	4-5 days post 1st vac	21-30 days post 1st vac	2-3 days post 2nd vac	4-5 days post 2nd vac	21-30 days post 2nd vac	90 – 100 days post 1st vac	121-131 days post 1st vac	151-161 days post 1st vac	182 – 192 days post 1st vac	216- 225 days post 1st vac	250- 259 days post 1st vac	284-293days post 1st vac	318-327 days post 1st vac	352-361 days post 1st vac	386-395 days post 1st vac
Assess SAEs, AESIs, medically attended AEs, AEs leading to withdrawal, and NOCD ^m	X			X			X	X	X	X	X	X	X	X	X	X	X
Assess relevant medications ⁿ	X			X			X	X	X	X	X	X	X	X	X	X	X
Study Termination ^o																	X
Footnotes:																	
a. See sections 3.2.1 and 12.2			d. See sections 3.2.5.1 and 4.3			j. See section 3.2.5.3											
b. See section 6.2			e. See sections 4.1 and 4.2			k. See section 3.2.5.4											
c. See section 3.5			f. See sections 3.2.3 and 3.2.4			l. See section 3.4.1											
			g. See sections 3.1 and 3.6			m. See sections 6.6.1, 6.6.1.1, 6.6.2 and 6.6.3											
			h. See sections 4.1, 4.2 and 4.4			n. See section 5.4											
			i. See sections 3.2.5.2, 5.1 and 5.3			o. See section 3.2.5.8											

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
AP	(Statistical) Analysis Plan
CDMS	Clinical Data Management and Standards
CBER	Center for Biologics Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
CI(s)	Confidence Interval(s)
CRF	Case Report Form
EC	Ethics Committee
EDC	Electronic Data Capture
EDT	Electronic Data Transfer
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
FDA	Food and Drug Administration
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HI	Haemagglutination Inhibition
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IRT	Interactive Response Technology
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NOCD	New Onset of Chronic Disease
PPS	Per Protocol Set
SAE	Serious Adverse Event
SDA	Source Data Agreement
SOC	System Organ Class
SOP	Standard Operating Procedure
VSAE	Vaccine Serious Adverse Event
WHO	World Health Organization

1.0 BACKGROUND AND RATIONALE

Study V89_18 is part of a program funded by the US Department of Health and Human Services (DHHS) to develop a cell culture-derived influenza vaccine in accordance with the US Pandemic Preparedness Plan. This DHHS contract supports the evaluation of safety and effectiveness of the MF59™ adjuvant in a cell culture-derived pandemic influenza vaccine. If there is an H5N1 virus pandemic, the strategy of having a pre-pandemic H5N1 vaccine, even if incompletely matched to the pandemic H5N1 virus and perhaps giving relatively low protection, may prevent more infections and deaths than waiting for completely matched pandemic H5N1 vaccines to be produced and distributed ([Ferguson 2006](#)).

An influenza pandemic occurs when a novel influenza virus emerges against which the vast majority of the world's population has no immunity. Outbreaks of influenza in animals, especially when they occur during annual outbreaks in humans, result in the merging of animal and human influenza viruses and increase the chances of a pandemic.

This phenomenon has been observed only with influenza A viruses and results from the emergence of a new antigenic variant (antigenic shift) caused by substitution of the hemagglutinin antigen on the surface of the virus, with or without a concomitant change in neuraminidase, the other major surface antigen ([Treanor 2004](#)). If such a virus demonstrates the ability to transmit efficiently from person to person, the result is a global outbreak of disease that affects a high percentage of individuals in a short period of time and is likely to cause substantially increased morbidity and mortality in all areas of the world.

Over 40 million people are estimated to have succumbed to the most devastating influenza pandemic in 1918, the so-called "Spanish flu". The "Asian flu" of 1957 was responsible for about 70,000 deaths in the US alone. As the volume and speed of international travel has increased during the 20th and early 21st centuries, successive pandemics have spread worldwide in ever decreasing amounts of time. On June 11, 2009, WHO officially declared the start of the 2009 influenza pandemic, the first influenza pandemic in more than 4 decades. Unlike previous pandemics, some of the population had prior infection experience with this subtype. In contrast to seasonal influenza viruses, the 2009 H1N1 virus disproportionately affected younger populations and its virulence was similar to that of seasonal influenza viruses. In August 2010 WHO officially declared the end of the pandemic.

Vaccines are the main prophylactic measure against pandemic influenza and have an important role in pandemic preparedness plans worldwide. For a pandemic, a specific monovalent vaccine against the new pandemic virus strain will have to be developed, approved, and produced in very large quantities ([Fedson 2003](#)). Preliminary findings have

identified the H2, H5, H6, H7 and H9 subtypes of influenza A as those most likely to be transmitted to humans and therefore present a potential pandemic threat. Widespread circulation, pathogenicity and direct transmission of avian viruses to humans suggest that H5N1 has important pandemic potentialities, increased by the high case fatality rate.

In 1997, the first outbreak of highly pathogenic H5N1 avian influenza occurred in Asia (Hong Kong). This strain re-emerged in 2003 leading to worldwide concerns over the possibility of an H5N1 pandemic. According to WHO, 637 human cases of H5N1 infection have been identified from 2003 through August 2013 and 378 of these died, representing a case fatality rate of 59.3% (WHO 2013). Highly pathogenic avian influenza H5N1 viruses have not yet been detected among wild birds, domestic poultry, or humans in the United States.

The Sponsor is developing a monovalent inactivated influenza vaccine that is adjuvanted with MF59C.1 (MF59 proprietary adjuvant) and uses a surface antigen from a potential pandemic H5N1 virus strain candidate (A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain) to be tested in clinical studies. The MF59 adjuvant is an oil-in-water emulsion, composed mainly of squalene, which is an intermediate metabolite in the synthesis of cholesterol. This vaccine contains the same MF59 adjuvant [REDACTED]

FLUAD is currently licensed in many European countries and other countries worldwide for active immunization against influenza in elderly (65 years of age and over), especially for those with an increased risk of associated complications (i.e., individuals affected by underlying chronic diseases including diabetes, cardiovascular, and respiratory diseases). FLUAD is not licensed in the United States. Since product launch in 1997 in Italy, approximately [REDACTED] of FLUAD have been distributed worldwide.

Previous clinical experience suggests that two doses of non-adjuvanted H5N1 influenza vaccine with 90µg of strain-specific hemagglutinin (HA) are necessary to achieve protective levels of antibodies in unprimed, immunologically naïve individuals, which represents 6 times the normal 15µg/dose required for the interpandemic seasonal influenza vaccine (Treanor 2006). The use of an adjuvant, however, allows reducing the quantity of antigen per dose and would potentially lead to increased vaccine production capacities (Nicholson 2001). Although it is unlikely that a single dose of the H5N1 vaccine would be enough to achieve protective antibody levels even if the adjuvant is added.

The observation of cross-reactive immune responses after vaccination with MF59-adjuvanted H5N1 vaccines is potentially of great interest for the development of pre-pandemic vaccines, since it suggests that the use of the MF59 adjuvant may ameliorate protection from H5N1 infection, even for H5N1 virus strains that have undergone antigenic drift.

Currently licensed pandemic and pre-pandemic vaccines are produced using the conventional egg-based manufacturing process. However, in the event of a new pandemic, there will be a dramatic increase of vaccine demand that will overwhelm global production capacity. Moreover, any condition that compromises availability of eggs (e.g. bird flu pandemic) will dramatically reduce production of influenza vaccines. Thus, increasing the availability of influenza vaccine to prevent the next pandemic can be viewed as largely dependent on developing methods of producing influenza vaccines that can avoid the use of eggs as the primary production substrate. Clinical development of the cell culture-derived, MF59-adjuvanted H5N1 (aH5N1c) vaccine helps address the medical need of a safe and effective pandemic and pre-pandemic vaccine.

The cell culture-derived process is not subject to the limitations of egg-based production and will therefore be a major public health benefit in the scenario of a pandemic.

Efficacy and safety of the aH5N1c vaccine needs to comply with stringent regulatory standards for new vaccines. The Sponsor has already conducted a Phase 1/2 dose ranging clinical study (V89P1) under US-IND in healthy adults 18 to 40 years of age, using 12 different adjuvanted or non-adjuvanted formulations of the cell culture-derived H5N1 vaccine. Immunogenicity results from this study assessed by cell culture-derived HI, SRH and MN assay demonstrated that formulations without the adjuvant did not meet any of the CBER criteria, while all formulations with 0.125mL (50%) or 0.25mL (100%) doses of the standard dose of MF59 achieved the CBER seroconversion criterion 3 weeks after the second dose. No formulation achieved the CBER criterion of HI titer $\geq 1:40$ as the study was not powered for this, although point estimate levels approached the 70% cut-off. Furthermore, for all pairwise comparisons at the same antigen level, formulations with adjuvant were more immunogenic 3 weeks after the second vaccination than those without adjuvant, when assessed as geometric mean titers (GMTs) and seroconversion rates ($p < 0.001$). These immunogenicity data clearly showed that the MF59 adjuvant is an essential component of the aH5N1c vaccine formulation, and is necessary to elicit an adequate immune response.

Laboratory safety tests performed in a subset of 120 subjects from the V89P1 Phase 1/2 study did not raise any safety concerns. The aH5N1c vaccines were well tolerated in healthy adults (18-40 years of age), and reactogenicity and safety results of all 12 vaccine groups in study V89P1 were similar. No dose-dependent safety issues or vaccine-related long-term effects on safety were observed in this dose-ranging Phase 1/2 study.

Three Phase 2 studies in separate populations groups have been performed evaluating “low dose” (3.75 μ g HA of H5N1 with 0.125mL MF59) and “high dose” (7.5 μ g HA of H5N1 with 0.25mL MF59) formulations of aH5N1c:

- V89_04 - adults 18 to <65 years of age

- V89_11 – children and adolescents 6 months to <18 years of age
- V89_13 – adults \geq 65 years of age

Results from these Phase 2 studies have provided data to support selection of the high dose aH5N1c vaccine in the V89_18 Phase 3 study. The proposed study is designed to evaluate the safety, immunogenicity and lot-to-lot consistency of aH5N1c vaccine and provide the support required for registration of the product.

A comprehensive review of aH5N1c is contained in the Investigator's Brochure supplied by the Sponsor; this document should be reviewed prior to initiating the study.

The trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirement(s).

2.0 OBJECTIVES

2.1 Primary Objectives

Co-Primary Immunogenicity Objectives

To demonstrate lot-to-lot consistency across three consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of GMTs of hemagglutination inhibition (HI) antibody responses to the H5N1 vaccine strain 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age.

After lot-to-lot consistency is demonstrated, the populations of all H5N1 c vaccine recipients will be pooled in order to evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER guidance 3 weeks after the second vaccine administration (Day 43) as measured by age cohort and by strain-specific HI assay.

Primary Safety Objective

To evaluate the safety and tolerability of aH5N1c vaccine and placebo in healthy adult subjects ≥ 18 years of age.

2.2 Secondary Objectives

Secondary Immunogenicity Objectives

After lot-to-lot consistency is demonstrated, the populations of vaccine recipients administered aH5N1c lots will be pooled, and the following objectives assessed:

- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CHMP recommendations (CHMP 2003, CHMP 2006) 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age by age cohort⁴, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER and CHMP recommendations 3 weeks after the first vaccine administration (Day 22), in subjects ≥ 18 years of age by age cohort⁴, as measured by strain-specific HI assay.

⁴ Both CBER and CHMP criteria will be evaluated separately for each age cohort (18 to <65 years and ≥ 65 years, and 18 to <60 years and ≥ 60 years respectively).

- To evaluate immune responses to aH5N1c vaccine 6 months after first vaccine administration (Day 183) in subjects ages ≥ 18 years of age, by age cohort, as measured by strain-specific HI assay.

3.0 STUDY DESIGN AND INVESTIGATIONAL PLAN

3.1 Overview of Study Design

This is a Phase 3 stratified, randomized, observer-blind, multi-center, placebo-controlled study to evaluate safety, immunogenicity and lot-to-lot consistency of aH5N1c in healthy adult subjects ≥ 18 years of age.

Staff participating in this multicenter trial will be trained in a uniform fashion and sites will be monitored to ensure consistency in study execution across all centers.

A total of approximately 3,192 subjects will be recruited and randomized at in 1:1:1:1 ratio, stratified by site and age, to receive one of 3 lots of aH5N1 or placebo (Groups A, B, C, and D). The design is summarized in [Table 3.1-1](#).

Table 3.1-1: Study Design

Treatment Arm	Schedule of Vaccine Administration	Total Enrolled	Enrolled by Age Group	
			Age 18 to <65 yrs	Age ≥ 65 yrs
Group A: aH5N1c lot #1	Day 1, Day 22	798	399	399
Group B: aH5N1c lot #2	Day 1, Day 22	798	399	399
Group C: aH5N1c lot #3	Day 1, Day 22	798	399	399
Group D: Placebo	Day 1, Day 22	798	399	399

The 2 doses of vaccine will be administered 3 weeks apart, on Day 1 and Day 22. The co-primary immunogenicity analysis will be based on HI antibody titers collected 3 weeks after the second vaccine administration, on Day 43. Durability of HI antibody response will be evaluated 6 months after the first vaccine administration, i.e. Day 183.

The study duration for each subject will be of approximately 13 months, or approximately 12 months after the second vaccine administration. The study requires that the site be in contact with the subject 17 times over the course of the trial, including 5 clinic visits, 4 reminder calls and 8 safety calls during Treatment and Follow-up Periods.

- Treatment Period: Day 1 through Day 42, which includes 2 clinic visits and 4 reminder calls.

- Follow-up Period: Day 43 through Day 387, which includes 3 clinic visits and 8 safety calls.

After signing of the informed consent form (ICF), eligible subjects will be enrolled in the study after undergoing a review of their medical history and a physical examination,

Vaccine administration will be performed in an observer-blind manner (i.e. efforts should be taken to shield the subject and blinded study team members from viewing vaccine administration).

After each vaccination, all subjects will remain under medical supervision at the study site for at least 30 minutes to be monitored and evaluated for AEs. Subjects will be instructed on the measurement of local and systemic solicited AEs, including body temperature, and on the completion of the diary cards.

The diary cards will collect solicited and unsolicited AEs, and medication/vaccinations given from Day 1 to Day 7 (inclusive) and from Day 22 to Day 28 (inclusive). From Day 8 to 21 and from Day 29 to 42, only unsolicited AEs, solicited AEs that continue beyond Day 7 or 28, respectively, and medications/ vaccinations will be collected at the time of return to the clinic on Day 22 or 43, respectively.

In addition to these safety data, from Day 1 through Day 387 (inclusive) serious adverse events (SAEs), adverse events of special interest (AESIs), new onset of chronic disease (NOCD), AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events, and all vaccinations will be recorded. For full list of AESIs see [appendix A](#). These data will be captured through the diary card, by interview of the subject, and by review of available medical records.

Blood will be drawn from each subject for immunogenicity assessments before each vaccination (Day 1 and Day 22) and also drawn at clinic visits on Day 43 and Day 183. Blood drawn will be evaluated for antibody responses as measured by HI antibody responses and possibly also microneutralization (MN) antibody responses.

An interim analysis of immunogenicity will be performed for all subjects through the Day 43 visit. Site staff and Sponsor personnel involved in study conduct will remain blinded to treatment assignments for this interim analysis.

The final analysis will be performed when all data up to the study end (Day 387) are available. Analysis will be done on fully unblinded data by the Sponsor and all data will be presented in the final, cumulative study report. A Data Monitoring Committee (DMC) will not be used for this study.

To maintain the observer-blind design of the study for the two vaccine doses (Day 1 and Day 22), the roles and responsibilities of blinded and unblinded team members will be defined and maintained throughout the study. Safety assessments and study related procedures and monitoring thereof must be performed by blinded team members.

3.1.1 Study Period

For the purpose of this protocol, end of study is defined as the completion of the Last Subject Last Visit (LSLV).

3.2 Study Visit Procedures

The following sections provide an overview of the procedures to be followed in the Treatment and Follow-up periods of this study for participating subjects.

3.2.1 Informed Consent

"Informed consent" is the voluntary agreement of an individual to participate in research. Consent must be given with free will of choice, and without undue inducement. The individual must have sufficient knowledge and understanding of the nature of the proposed research, the anticipated risks and potential benefits, and the requirements of the research to be able to make an informed decision.

If a subject is unable to read, an impartial witness should be present during the entire informed consent discussion. An impartial witness is defined as a person who is independent from trial conduct, who cannot be unfairly influenced by those involved with the trial, who attends the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the ICF and any other written information supplied to the subject. After the written ICF and any other written information to be provided to the subject, is read and explained to the subject and after the subject has verbally consented to the subject's participation in the trial and, if capable of doing so, has signed and personally dated the ICF, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the ICF and any other written information was accurately explained to, and apparently understood by, the subject and that informed consent was freely given by the subject.

3.2.2 Screening Procedures

After an individual has consented to participate in the study and ICF is signed, a Subject Number will be assigned and screening procedures carried out that should include the following:

1. Obtain and record demographic data, medical history and concomitant medications (see [sections 5.4](#) and [6.2](#)).
2. Privately review with females of childbearing potential their ability to become pregnant. Also verify that they have used a reliable birth control method for at least two months prior to study entry. Confirm their commitment to practice appropriate birth control until 3 weeks after the second vaccine administration (i.e., up to Day 43). Determine the date of the subject's last menstrual period. A pregnancy test will be performed on all female subjects of childbearing potential (see [section 3.5](#)). If the pregnancy test is positive or indeterminate, the subject must not be enrolled and vaccinated.
3. Perform a review of systems by interview that queries the subject as to any complaints the subject has experienced across each organ system.
4. Perform a general physical examination (see [section 6.2](#)).
5. Confirm that the individual meets ALL inclusion and NO exclusion criteria.
6. In the event that the individual is determined ineligible for study participation, he/she is considered a "screen failure". The reason for screen failure must be documented in the Screening and Enrollment Log.
7. If the individual is determined to be eligible for the study, he/she should be enrolled into the study as described in [section 3.2.3](#).

3.2.3 Enrollment

After signing the informed consent form, if an individual is determined to be eligible for study participation, the investigator will enroll the subject and enter the Subject Number into an Electronic Data Capture (EDC) system.

3.2.4 Randomization

Enrolled subjects will be randomly assigned to the study groups, either one of 3 lots of aH5N1c or placebo, in a pre-specified ratio of 1:1:1:1. Randomization will be stratified by center, and by age cohort. At randomization, a unique code for the assigned treatment study materials will be supplied to the site, using an Interactive Response Technology (IRT) system. A validated randomization system will be used.

After randomization, the Subject Number continues to be used for subject identification for the duration of the study.

If for any reason, after signing the informed consent form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure and the

early termination study procedures must be applied. The reason for all randomization failures should be recorded in the Screening and Enrolment Log and in the source document as specified in the Source Data Agreement (SDA). The information on subjects who are randomization failures should be kept distinct from subjects who are screen failures, as described in section 3.2.2.

If for any reason, after randomization the subject fails to undergo treatment, this is an Early Termination and the reason should be recorded in source document as specified in the SDA. The information on these Early Termination subjects should be kept distinct in the source documentation from randomization failures.

3.2.5 Visit Procedures

3.2.5.1 Pre-vaccination procedures

The following procedures should be carried out at the clinic visits before the vaccinations on Day 1 and Day 22 are performed:

1. Before or on Day 1 clinic visit only:
 - a. Obtain informed consent (see [sections 3.2.1 and 12.2](#))
 - b. Perform screening procedures (see [section 3.2.2](#))
2. On the Day 22 visit, the diary card will be reviewed. Please see [section 3.4.1](#) for additional guidance on diary card review.
3. Perform a review of systems by interview that queries the subject as to any complaints the subject has experienced across each organ system (see [section 6.2](#)).
4. Perform a general physical examination (see [section 6.2](#))
5. Privately review with females of childbearing potential their ability to become pregnant. Also verify that they will use/continue to use a reliable birth control method until 3 weeks after the second vaccine administration. (see [section 3.2.2](#)). Determine the date of the subject's last menstrual period. A pregnancy test will be performed on all female subjects of childbearing potential. If the pregnancy test is positive or indeterminate, the subject must not be vaccinated (see [sections 3.5, 3.8 and 6.6.4](#)).
6. On Day 1 clinic visit only:
 - a. Enroll the subject (see to [section 3.2.3](#))
 - b. Randomize the subject (see to [section 3.2.4](#))
7. Body temperature (preferably oral) must be measured on the day of vaccination. If it is ≥ 38.0 °C (≥ 100.4 °F), vaccination must be postponed until three days after the

fever has resolved. Vaccination is also postponed for any clinically significant active infection (see [section 4.3](#)).

8. The use of analgesics and/or antipyretics is strongly discouraged within 24 hours prior to vaccination (see [section 5.4](#)).
9. Prior to study vaccinations, draw blood from the subject for serology testing. Details regarding the volume of blood and testing to be performed are in [section 3.6.1](#).

3.2.5.2 Vaccination procedures

Vaccinations will be performed on Day 1 and Day 22.

On Day 1 only, after confirming eligibility, enrolling, randomizing and performing the pre-vaccination procedures for the subject, perform vaccination of the subject according to the assigned study vaccine and according to the procedures described in [section 5.3](#) and observing the blinding procedures described in [section 3.3](#).

At the visit on Day 22, confirm that the subject does not meet any criteria for delaying or cancelling additional study vaccinations, as described in [section 4.3](#) and [section 4.4](#) of the protocol before performing the vaccination according to the procedures described in [section 5.3](#) and [3.3](#).

3.2.5.3 Post-vaccination procedures

The following post-vaccination procedures will be performed on Day 1 and Day 22:

1. Careful training of the subject on how to measure solicited local AEs, body temperature and how/how often to complete the diary card is crucial. Training should be directed at the individual who will perform the measurements of the local AEs and those who will enter the information into the diary card. This individual may not be the subject, but if a person other than the subject enters information into the diary card, this person's identity must be documented in the diary card and this person must receive training on its completion. Training of the subject on how to measure an injection site AEs should be performed while the subject is under observation after vaccination. Diary card instruction must include the following:
 - a. The subject must understand that timely completion of the diary card on a daily basis is a critical component to study participation. The subject should also be instructed to write clearly and to complete the diary card in pen. Any corrections to the diary card that are performed by the person completing the diary card should include a single strikethrough line with a brief explanation for any change. No changes can be made to the diary card when it is returned to the clinic.

- b. Starting on the day of vaccination, the subject will check in the evening for specific types of reactions at the injection site (solicited local AEs), any specific generalized symptoms (solicited systemic AEs), body temperature (taken preferably orally), any other symptoms or change in the subject's health status, and any medications/vaccinations received (excluding vitamins, minerals and homeopathic treatments). These solicited AEs, unsolicited AEs and body temperature will be recorded in the "six hour" location on the diary card.
 - c. Body temperature measurement is to be performed using the thermometer provided by the site. If the subject feels unusually hot or cold during the day, the subject should check body temperature. If the subject has fever, the highest body temperature observed that day should be recorded on the diary card. If the temperature is $< 35.5^{\circ}\text{C}$ ($< 95.9^{\circ}\text{F}$), the subject should be instructed to repeat the measurement to ensure it is correct. The measurement of solicited local AEs is to be performed using the ruler provided by the site. The collection of body temperature, solicited local AEs, solicited systemic AEs will continue for a total of 7 days on the diary card. If the temperature is $\geq 38.0^{\circ}\text{C}$ ($\geq 100.4^{\circ}\text{F}$) on Day 7, the subject should be instructed to continue to measure the temperature each day, as described above, until it returns to $< 38.0^{\circ}\text{C}$ ($< 100.4^{\circ}\text{F}$) for the complete day. The recording of unsolicited AEs and medications/vaccinations will continue for 21 days, or up to the evening prior to the next clinic visit.
2. After vaccination, the subject will be observed for at least 30 minutes including observation for AEs, and body temperature measurement. Please take the opportunity to remind the subject how to measure solicited AEs and body temperature as part of this observation period. Record all safety data collected in the subject's source documents.
 3. Schedule the next study activities, reminder calls and clinic visit, with the subject.
 4. Remind the subject to complete the diary card daily and to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization.

3.2.5.4 Reminder calls

Reminder calls will be performed approximately 2 and 4 days after each vaccination on Days 3, 5, 24, and 26 (see [Table 2, Time and Events Table](#)).

Reminder calls or alerts are not intended to be an interview for collection of safety data. If the subject wishes to describe safety information, this information should only be collected by a healthcare professional at the site, and the safety data described must be written down in the subject's medical chart.

The purpose of this call is to remind the subject about completion of the diary card. The call follows the reminder telephone call script provided to the site. The subject should be reminded to contact the site via the telephone number provided in the informed consent to discuss medical questions.

3.2.5.5 Clinic visits after vaccination

Clinic visits that do NOT include vaccine administration will be performed approximately 2, 6, and 13 months after vaccination 1 on Day 43, 183, and 387 (see [Table 2, Time and Events Table](#)).

At the clinic visit on Day 43 the diary card will be reviewed. Please see [section 3.4.1](#) for additional guidance on diary card review.

1. At clinic visits where a diary card is not brought back and reviewed (Days 183 and 387) interview the subject guided by a script, to obtain information relating to unsolicited AEs including SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events, and all vaccinations (see [section 5.4](#)). All safety information described by the subject must be written down in the source documents and not written on the script used for the interview (see [section 3.4.1](#)).
2. Record any relevant medical history as needed (see [section 6.2](#)).
3. Perform a review of systems by interview that queries the subject as to any complaints the subject has experienced across each organ system.
4. Perform a symptom-directed physical examination (see [section 6.2](#)).
5. At clinic visit on Day 43, (as applicable for females of childbearing potential, see [section 3.5](#)), determine the date of the subject's last menstrual period. A pregnancy test will be performed on all female subjects of childbearing potential.
6. At clinic visits on Day 43 and 183, blood will be drawn from the subject for serology testing. Details regarding the volume of blood and testing to be performed can be found in [section 3.6.1](#).
7. Schedule the next safety call with the subject.
8. Remind the subject to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

3.2.5.6 Safety calls

Safety calls will be performed approximately 3, 4, 5, 7, 8, 9, 10.5, and 12 months after the first vaccination on Day 91, 122, 152, 217, 251, 285, 319, and 353 (see [Table 2, Time and Events Table](#)).

1. Safety calls should be done by a trained healthcare professional. These calls will follow a script which will facilitate the collection of relevant safety information. The subject will be interviewed according to the script, to obtain information relating to unsolicited AEs including SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events, and all vaccinations. All safety information described by the subject must be written down in the source documents and not written on the script used for the telephone call (see [section 3.4.1](#)).
2. Record any relevant medical history as needed (see [section 6.2](#)).
3. Schedule the next study activity (clinic visit or safety call) with the subject.
4. Remind the subject to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization.

3.2.5.7 'For cause' visits

Not applicable for this study.

3.2.5.8 Termination visits

The termination visit will normally occur on approximately Day 387. **For visit procedures to be performed for a subject whose planned study participation ends prematurely, please see [section 3.8](#).**

1. Interview the subject, guided by a script, to obtain information relating to unsolicited AEs including SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events, and all vaccinations (see [section 5.4](#)). All safety information described by the subject must be written down in the source documents and not written on the script used for the interview (see [section 3.4.1](#)).
2. Record any relevant medical history as needed (see [section 6.2](#)).
3. Perform a review of systems by interview that queries the subject as to any complaints the subject has experienced across each organ system.
4. Perform a symptom-directed physical examination (see [section 6.2](#)).

5. Let the subject know when information relating to the subject's participation in the study may be available (e.g. study results, treatment assignments). Also discuss how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject chooses to share this information.
6. Complete the termination electronic case report form (eCRF) page and this will mark the completion of the subject's participation in the study.

3.3 Blinding Procedures

The trial is designed as an observer-blind study. During the Treatment Phase of the study (Day 1 through Day 43), designated unblinded nurse(s), physician(s), or other qualified personnel will be responsible for administering the study vaccines to the subjects. They will be instructed not to reveal the identity of the study vaccines either to the subject or the investigative site personnel (i.e., investigator and study nurse) involved in the monitoring of conduct of the trial, except in an emergency.

The designated unblinded nurse(s) or physician(s) will not take part in evaluating the subject(s) for safety or collect study data after the vaccinations. Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In such instance of medical emergency, every effort should be made to contact the Sponsor prior to unblinding. If unblinding should occur (by either accidental unblinding or emergency unblinding for a serious adverse event) prior to completion of the study, the investigator must promptly contact the Sponsor and document the circumstances on the appropriate forms. Instructions regarding emergency unblinding will be provided to the investigator.

Study vaccine allocations (first and second vaccinations at Day 1 and Day 22) will not be available to the investigator or personnel monitoring the trial until after the completion of the trial and final review of Day 387 data.

A formal interim analysis of immunogenicity will be carried out by an independent statistician and programmer (to preserve the blind) after Day 43 immunogenicity data are available for all subjects (excluding those lost to follow-up or withdrawn for other reasons). The results of that interim analysis will have limited distribution to internal personnel and appropriate external public health or regulatory agencies, who are not involved in the ongoing conduct of the trial. Investigators, other site, CRO and Sponsor personnel, and the serology laboratory) will remain blinded to subjects' individual study arm assignment until final data from all termination visits are locked.

3.4 Data Collection

3.4.1 Data collected from subjects

All data collected from subjects and provided to the Sponsor for analysis must be stripped of any identifiers that reveal the identity of that individual (beyond the use of subject ID, as described in [section 3.2.3](#)).

The use of any written or verbal information identifying the subject such as name, initials, photos or testimonials, requires separate and appropriate documented consent from the subject.

Diary cards will be the only source document allowed for solicited systemic and local AEs (including body temperature measurements), starting after the initial 30-minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the diary cards:

1. No corrections or additions to the diary card will be allowed after it is delivered to the site.
2. Any blank or illegible fields on the diary card must be described as missing in the eCRF.
3. The site must enter all readable entries in the diary card into the eCRF, including those values that may be biologically implausible (e.g. body temperature: 400°C).
4. Any illegible or implausible data should be reviewed with the subject. For example, if the subject with a body temperature of 400°C describes that the body temperature was actually 40°C on the day in which body temperature: 400°C was written into the diary card, this fever of 40°C should be described in the source documents and reported as a verbally reported (unsolicited) AE in the AE eCRF.
5. Any new safety information described at the visit (including a solicited AE not noted in the diary card) must NOT be written into the diary card and should be described in the source documents and reported as a verbally reported (unsolicited) AE in the AE eCRF.

3.4.2 Electronic and Paper Case report forms

Paper (VSAE form, pregnancy follow-up CRF) and electronic CRFs will be used in this study. Data will be entered onto electronic and paper CRFs by the investigator and/or the investigator's dedicated site staff. See [section 9.1](#) for more details.

3.5 Pregnancy Testing

Urine pregnancy testing will be performed at the sites on all women of childbearing potential. Results of the pregnancy tests must be recorded in the source documents and CRFs.

Note: a female of childbearing potential is defined as a post onset menarche and premenopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (i) tubal ligation at least one year earlier, or (ii) bilateral oophorectomy or hysterectomy. Reliable birth control method is defined as hormonal (e.g. oral, injection, transdermal patch, implant, cervical ring), barrier (e.g. condom with spermicide or diaphragm with spermicide), intrauterine device (e.g. IUD), sexual abstinence or monogamous relationship with partner who has been vasectomized for six months or more prior to the subject's study entry.

3.6 Laboratory Assessments

3.6.1 Processing, Labeling and Storage of Serum Samples for Serology

Approximately 10 – 12 mL sample of blood will be drawn from all subjects at Day 1 and Day 22 before vaccination, and at Day 43 and Day 183. The blood volume will not exceed 12 mL at each time point in order to provide the necessary serum volume (approximately half of the blood draw volume) for the serology assays. All samples will be tested blinded to subject ID, visit numbers and treatment assignment.

For further details please see the Investigator Laboratory Manual which is located in the Investigator Site File.

Samples will be retained in accordance with regulatory guidance for retention of essential study documents as described in [section 10](#).

3.6.2 Safety Laboratory Assessments

No Safety Laboratory assessments are included for this study.

3.6.3 Cell Mediated Immunity Assessments

No Cell Mediated Immunity assessments are included for this study.

3.6.4 Culture/PCR/Genotyping Assessments

No Culture/PCR/Genotyping assessments are included for this study.

3.7 Stopping/Pausing Guidelines

There are no predetermined stopping rules other than circumstances for which subjects may not be eligible for additional study vaccinations as described in [section 4.4](#) or may be removed from the study according to investigator discretion as described in [section 3.8](#).

3.8 Premature Withdrawal and Early Study Termination

A subject may discontinue, or be discontinued from, study participation at any time prior to the last planned study visit. This is referred to as **premature withdrawal from the study** (see below for a description of withdrawal from study vaccine for subjects which refers to those subjects who do not receive additional vaccine doses but continue in the study for safety follow-up and/or other procedures). The reasons for premature withdrawal from the study include:

- Adverse event
- Death
- Withdrawal of consent
- Lost to follow-up
- Administrative reason
- Protocol deviation
- Other

NOTE: Before entering any alternate category as the reason for the subject's discontinuation from the study, the investigator should make every effort to investigate whether or not safety concerns (AE or death) may have been related to the subject's discontinuation from the study. If a safety concern has been associated with the subject's discontinuation, this must be described on the Termination eCRF page, even if it is not the primary reason for the subject's discontinuation.

For any subject withdrawing from study participation prior to the planned Termination visit, it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the AE eCRF page by indicating "Withdrawn from study due to AE".

For any subject withdrawn from study participation due to death, this should be noted on the Termination eCRF page and the associated SAE that led to the death must be reported.

The subject can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the subject is otherwise entitled. Reason for early termination should be deemed as “withdrawal of consent” if the subject withdraws from participation due to a non-medical reason (i.e., reason other than AE). Subjects should be encouraged to be followed for safety for the duration of the study. When a subject withdraws, or is withdrawn, from the study, the procedures described in [section 3.9, Early Termination Visit](#), should be completed if possible.

The date of termination is the date of the last contact (clinic visit or telephone) in which the subject’s health status was assessed or, in cases where the subject does not agree to any further safety follow-up, it is the date consent is withdrawn.

In sites in the United States, if a subject withdraws consent but does not revoke the Health Insurance Portability and Accountability Act authorization (HIPAA), the Sponsor will have full access to the subject’s medical records, including termination visit information. If a subject revokes only the HIPAA authorization, the Sponsor will have full access to all of the subject’s medical records prior to the date and time of written revocation.

For subjects who fail to show up for scheduled visits (clinic or telephone contacts), study staff are encouraged to make at least three documented attempts to contact the subject by telephone and at least one documented written attempt to contact the subject and encourage the completion of study termination procedures. These efforts to contact the subject should be recorded in the source documents. The termination date for the subject to be captured on the Termination eCRF page is the date of the last successful visit (clinic or telephone) with the subject.

For subjects who are withdrawn from the study due to Sponsor decision (e.g. meeting pre-specified withdrawal criteria or termination of study by the Sponsor), this reason should be noted in the Termination eCRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

In general, subjects associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the subject’s health, safety, or rights. For subjects who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Termination eCRF page. Any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization. This would **not** include any subject who became pregnant during study conduct despite contraception. See below for greater detail.

If a subject is withdrawn prematurely from the study for a reason other than those outlined above, this reason must be documented in the Termination eCRF page.

The act of withholding additional study vaccinations is referred to as ‘**withdrawal from study vaccination**’. Subjects may be withdrawn from study vaccination for several reasons including but not limited to: failure to meet criteria for revaccination (see [section 4.4](#)), or pregnancy (see [section 6.6.4](#)). **Subjects who are withdrawn from study vaccination should be encouraged to continue in the study for safety follow-up and other procedures as appropriate until the scheduled termination visit.** If the subject is withdrawn from study vaccination(s) due to AE, this event must be linked to the withdrawal from vaccination on the AE eCRF page.

The Sponsor or the investigator (following consultation with the Sponsor) has the right to discontinue this study at any time. If the clinical study is prematurely terminated, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate therapy and follow up for the subjects. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (study medication/vaccines, etc.) must be returned to the Sponsor.

Any subject who, despite the requirement for adequate contraception, becomes pregnant during the trial will not receive further vaccination but should be encouraged to continue participation in the study. The site should complete a Pregnancy Report CRF (initial report) as soon as possible (see [section 6.6.4](#)). If the subject withdraws from the study for any of the above categories except death, the site will obtain permission from the subject to continue to remain in contact with her until the outcome of the pregnancy is known, even if the outcome is not known until after the subject reaches the end of the routine study period.

Withdrawn subjects will not be replaced.

When a subject is withdrawn or withdraws from the study, the procedures described in [section 3.9](#) Early Termination Visit should be completed if possible.

3.9 Early Termination Visit

When a subject is withdrawn or withdraws from the study, the investigator will notify the Sponsor and, when possible, will perform the procedures listed below.

1. Collect diary card (if applicable).
2. Review the subject’s solicited and unsolicited safety data (if collection of these was in progress at the time of study termination).
3. Interview the subject, guided by a script, to obtain information relating to unsolicited AEs including SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, medically attended AEs, associated concomitant medications for any of these events,

and all vaccinations (see [section 5.4](#)). All safety information described by the subject must be written down in the source documents and not written on the script used for the interview (see [section 3.4.1](#)).

4. Record any relevant medical history as needed (see [section 6.2](#)).
5. Perform a review of systems by interview that queries the subject as to any complaints the subject has experienced across each organ system.
6. Perform a symptom-directed physical examination. See [section 6.2](#) or further detail.
7. Let the subject know when information relating to the subject's participation in the study may be available (e.g. study results, treatment assignments). Also discuss how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject chooses to share this information.
8. Complete the termination eCRF page and this will mark the completion of the subject's participation in the study.

4.0 SELECTION OF STUDY POPULATION

4.1 Inclusion Criteria

In order to participate in this study, all subjects must meet ALL of the inclusion criteria described.

1. Males and females 18 years of age or greater.
2. Individuals who have given written consent after the nature of the study has been explained according to local regulatory requirements.
3. Individuals in good health as determined by the outcome of medical history, physical examination and clinical judgment of the investigator.

4.2 Exclusion Criteria

In order to participate in this study, all subjects must meet NONE of the exclusion criteria described.

1. Individuals with behavioral or cognitive impairment.
2. Individuals with any progressive or severe neurologic disorder, seizure disorder, or history of Guillian-Barré syndrome.
3. Individuals who are not able to comprehend and to follow all required study procedures for the whole period of the study.
4. Individuals with a history of illness or with an ongoing illness that, in the opinion of the Investigator, may pose additional risk to the subject if he/she participates in the study.
5. Individuals who have a suspected or confirmed diagnosis of any AESI (see [Appendix A](#)).
6. Individuals with known or suspected impairment of the immune system, such as:
 - a. Use of systemic (oral or parenteral) corticosteroids for more than 14 consecutive days within 60 days prior to Day 1 (and up to 3 weeks after second vaccination). Use of inhaled, intranasal or topical corticosteroids is allowed.
 - b. Receipt of cancer chemotherapy within 5 years prior to Day 1.
 - c. Receipt of immunostimulants or immunosuppressives within 60 days prior to Day 1
 - d. Known human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS).

- e. Receipt of parenteral immunoglobulin preparation, blood products, and/or plasma derivatives within 3 months prior to Day 1 or planned during the full length of the study.
7. Individuals who are pregnant or breastfeeding. Female subjects of childbearing potential must have a negative pregnancy test prior to study vaccines being administered.
8. Females of childbearing potential⁵ who refuse to use an acceptable method of birth control⁶ from first vaccine administration (Day 1) to 3 weeks after the second vaccine administration (Day 43), and/or, if sexually active, who have not used a reliable birth control method for at least two months prior to study entry.
9. Individuals who are allergic to any of the vaccine components as outlined in the current Investigational Brochure.
10. Individuals who have had a malignancy (excluding nonmelanotic skin cancer) or lymphoproliferative disorder within the past 5 years from Day 1.
11. Individuals participating in any clinical trial with another investigational product 30 days prior to first study visit or intent to participate in another clinical study at any time during the conduct of this study.
12. Individuals with a body temperature ≥ 38.0 °C (≥ 100.4 °F) or any acute illness within 3 days of intended study vaccination.
13. Individuals who have had a previous confirmed or suspected illness from avian flu caused by an H5N1 virus.
14. Individuals who have received any prior H5N1 vaccine.
15. Individuals who received any type of influenza vaccine (e.g., “seasonal”) within 7 days prior to enrolment in this study or who are planning to receive any type of influenza vaccine within 7 days (before or after) from the study vaccines.
16. Individuals who received any other licensed vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this study or who are

⁵Female of childbearing potential is defined as a post onset menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, or (3) bilateral oophorectomy or hysterectomy.

⁶Reliable birth control method is defined as sexual abstinence, hormonal (e.g., oral, injection, transdermal patch, implant, cervical ring), barrier (e.g., condom with spermicide or diaphragm with spermicide), intrauterine device (e.g., IUD), or monogamous relationship with partner who has been vasectomized for 6 months or more prior to the Subject’s study entry.

planning to receive any (non-influenza) vaccine within 28 days (before or after) from the study vaccines.

17. Individuals who are research staff involved with the clinical study or family/household members of research staff.
18. Individuals with a BMI > 35 kg/m².
19. Individuals with a history of drug or alcohol abuse within the past 2 years.
20. Individuals who had household contact with and/or intimate exposure to an individual with culture-proven H5N1 infection, or exposure to infected household poultry or contaminated environments with sick and dead poultry, within 60 days prior to enrolment.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (e.g., body temperature elevation or recent use of excluded medication or vaccine). Under these circumstances, a subject may be considered eligible for study enrollment if the appropriate window for delay has passed, inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

4.3 Criteria for Delay of Vaccination and/or Blood Sampling

After enrollment, subjects may encounter clinical circumstances that warrant a delay in subsequent study vaccination. These situations are listed below. In the event that a subject meets a criterion for delay of vaccination, the subject may receive study vaccination once the window for delay has passed as long as the subject is otherwise eligible for study participation.

- Individuals who have experienced a temperature ≥ 38.0 °C (≥ 100.4 °F) and/or any acute illness within 3 days of intended study vaccination.
- Individuals who have received any type of influenza vaccine (e.g., “seasonal”) within 7 days of intended study vaccination
- Individuals who have received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) of intended study vaccination.

4.4 Criteria for Repeat Vaccination in the Study

There are also circumstances under which repeat vaccination is a contraindication in this study. Prior to receipt of second study vaccination, subjects must be evaluated to confirm

that they are eligible for subsequent vaccination. If subjects meet any of the criteria listed below, they should not receive additional vaccinations.

- Subjects who experience any SAE judged to be possibly or probably related to study vaccine or non-study vaccines, including serious anaphylaxis or hypersensitivity reactions.
- Subjects who develop any new condition which, in the opinion of the investigator, may pose additional risk to the subject if he/she continues to participate in the study.

Subjects who meet any of these criteria must not receive further study vaccinations. However, these subjects should be encouraged to continue study participation, as discussed in [section 3.8](#).

5.0 TREATMENT OF SUBJECTS

All vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring. All vaccines associated with this study must be checked for expiration date prior to use. Expired vaccines must not be administered to subjects.

5.1 Study Vaccine

The term 'study vaccine' refers to those vaccines provided by the Sponsor, which will be evaluated as part of the study objectives. The study vaccine specific to this study are described below.

The vaccine used for this study is aH5N1c vaccine, a cell culture-derived, MF59-adjuvanted, monovalent inactivated subunit H5N1 (A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain) vaccine. Each dose contains 7.5 mcg H5 hemagglutinin + 0.25 mL MF59.

Placebo control will consist of 0.5 mL sterile saline (0.9% NaCl).

Subjects are randomly assigned in a 1:1:1:1 ratio to one of the following vaccination group(s):

- aH5N1c vaccine, lot 1 (Group A)
- aH5N1c vaccine, lot 2 (Group B)
- aH5N1c vaccine, lot 3 (Group C)
- Placebo (Group D)

Composition of the aH5N1c vaccine with 7.5 mcg H5N1 hemagglutinin antigen (HA) + 0.25 mL MF59 (approximately 0.5mL extractable volume) is presented in [Table 5.1-1](#) below.

Table 5.1-1: aH5N1c Investigational Vaccine Composition

Vaccine Components	Full dose vaccine formulation (0.5mL volume)
Influenza virus surface antigens (haemagglutinin and neuraminidase) ; A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain	██████ (in ██████ volume of vaccine formulation)
Adjuvant	MF59 content in ██████ volume: Squalene (██████) Polysorbate 80 (██████) Sorbitan Trioleate (██████) Sodium Citrate (██████) Citric Acid (██████)
Excipients	Excipients content in ██████ volume: Sodium Chloride (██████) Potassium Chloride (██████) - Potassium Dihydrogen Phosphate (██████) - Dihydrogen Phosphate Dihydrate (██████) Magnesium Chloride Hexahydrate (██████) Calcium Chloride Dihydrate (██████)
Buffer	Up to ██████, Water for Injection
Volume of Formulation	██████
Appearance	White homogenous liquid. Free from visible foreign particulates.
Vaccine Presentation	Prefilled Syringe, ██████ total extractable volume

5.2 Non-Study Vaccines

No non-study vaccines will be given.

5.3 Vaccines Preparation and Administration

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines will be administered only by personnel (unblinded) who are qualified to perform that function under applicable local laws and regulations for the specific study site.

The Day 1 and Day 22 vaccine accountability/ management will be carried out by unblinded site staff. All vaccine information will be stored in a secure place. Only unblinded site staff will have access to this information.

Monitoring responsibilities regarding vaccine accountability are covered in Section 5.5.

Detailed vaccine preparation and administration instructions will be provided to investigators in the Investigator Manual prior to study start.

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING STUDY VACCINE:

Prior to vaccination, subjects must be determined to be eligible for study vaccination and it must be clinically appropriate in the judgment of the investigator to vaccinate.

Eligibility for vaccination prior to first study vaccine administration is determined by evaluating the entry criteria outlined in protocol [section 4.1](#) , Inclusion Criteria and [4.2](#), Exclusion Criteria.

Eligibility for subsequent study vaccination is determined by following the criteria outlined in [section 4.3](#), Criteria for Delay of Vaccination.

Eligibility for non-study vaccines should be determined by the investigator, pending the review of the package insert of the relevant vaccine.

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines.

Standard immunization practices are to be observed and care should be taken to administer the injection intramuscularly. Before administering vaccine, the vaccination site is to be disinfected with a skin disinfectant (e.g., 70% alcohol). Allow the skin to dry. **DO NOT inject intravascularly** or intragluteally.

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccine administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

5.4 Prior and Concomitant Medications and Vaccines

Data on all medications (except vitamins, minerals and homeopathic treatments) that were taken by the subject up to 2 months prior to enrollment (including birth control) will be collected in the relevant Prior and Concomitant Medications eCRF.

All vaccinations received up to 12 months before enrollment should be recorded. The subject should be questioned regarding plans to receive an influenza vaccine over the next 12 months.

The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source documents and Concomitant Medications eCRF.

In addition, the following are considered prior medications for this protocol, and should be recorded in the Concomitant Medications eCRF if taken within the timeframe indicated in [section 4.0 Selection of Study Population](#): all medication/vaccines described in the inclusion and exclusion criteria of this protocol including:

- Cancer chemotherapy.
- Immunosuppressive agents.
- Systemic corticosteroids at any dose.
- Parenteral immunoglobulin preparation, blood products, and/or plasma derivatives.
- Any type of influenza vaccination (e.g., “seasonal”) or other vaccines.

Medications taken for prophylaxis are those intended to prevent the onset of symptoms. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

Concomitant medications include all medications (including vaccines) taken by/administered to the subject at and after enrolment and must be documented on the Concomitant Medications CRF.

When recording concomitant medications/vaccines, they should be checked against the study entry and continuation criteria in [section 4.0 Selection of Study Population](#) to ensure that the subject should be enrolled/continue in the study.

Subjects will be instructed to record on the appropriate section of diary cards all medication taken (in addition to the study vaccine) during that observation period.

From study entry through termination, concomitant medications will be recorded in the Concomitant Medications eCRF as outlined below:

- All medications, with the exception of vitamins, minerals and homeopathic treatments, will be recorded beginning from the time of each vaccination and continuing during the following 6 days (Day 1 to 7 and Day 22 to 28);
- Systemic corticosteroids at any dose, single or chronic use from Day 1 to 3 weeks after second vaccination (Day 43);

- All medications associated with AEs will be recorded from the time of each vaccination and continuing during the following three weeks (Day 1 to Day 43);
- Medications associated with SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, and medically attended AEs will be recorded from Day 1 to Day 387 (study termination).
- All non-study vaccinations received between Day 1 and Day 387 (study termination).

5.5 Vaccine Supply, Labeling, Storage, and Tracking

The Sponsor will ensure the following:

- Supply the study vaccine(s)
- Appropriate labeling of all study vaccines provided that complies with the legal requirements of each country where the study is to be performed

The investigator must ensure the following:

- Acknowledge receipt of the study vaccines by a designated staff member at the site, including confirmation that the vaccines:
 - were received in good condition
 - remained within the appropriate temperature range during shipment from the Sponsor to the investigator's designated storage location
 - have been confirmed by the Sponsor as authorized for use
- Proper storage of the study vaccines, including:
 - storage in a secure, locked, temperature-controlled location
 - proper storage according to the instructions specified on the labels
 - appropriate record keeping and inventory of the study vaccines, including regular documentation of adequate storage temperature
- Appropriate use of the study vaccines, including:
 - use only in accordance with the approved protocol
 - proper handling, including confirmation that the vaccine has not expired prior to administration
 - appropriate documentation of administration of vaccines to study subjects including:
 - date, dosage, batch/serial numbers, expiration dates, unique identifying

numbers assigned to subjects and study vaccines, and time of vaccine administration. This information will be maintained in an accountability log that will be reviewed by the site monitor.

- proper reconciliation of all study and non-study vaccines received from the Sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines (and volume thereof) were administered to subjects, which vaccines were destroyed at the site, and which vaccines were returned to the Sponsor, as applicable.
- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, return or destruction, including documentation of:
 - copy of the site's procedure for destruction of hazardous material
 - number of doses destroyed, date of destruction, destruction code (if available), method of destruction, and name of individual performing destruction

Vaccines that have been stored differently from the manufacturer's indications **must not** be used unless the Sponsor provides written authorization for use. In the event that the use cannot be authorized, the Sponsor will make every effort to replace the vaccine supply. All vaccines used in conjunction with this protocol must be stored separately from normal hospital/practice stocks to prevent unintentional use of study vaccines outside of the clinical trial setting.

Monitoring of vaccine accountability will be performed by the study monitor during site visits and at the completion of the study.

At the conclusion of the study, and as appropriate during the course of the study, the investigator must return to the Sponsor or destroy (upon approval from Sponsor) all unused study vaccines, packaging and supplementary labels.

6.0 MEASUREMENTS

6.1 Appropriateness of Measurements

The measures of immunogenicity used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

The measures of safety used in this study are routine clinical procedures. They include a close vigilance for, and stringent reporting of, selected local and systemic AEs routinely monitored in vaccine clinical trials as indicators of reactogenicity.

6.2 Demographics, Medical History and Physical Examination

Prior to study enrollment, **demographic** data will be collected from the subject, including: age, sex, race, ethnicity, height, weight and prior vaccination against influenza (in the last 12 months).

Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications (see [section 5.4](#)), and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an AE that occurs during study participation, if it represents an exacerbation of an underlying disease/preexisting problem.

A **review of systems** is a structured interview that queries the subject as to any complaints the subject has experienced across each organ system. This will be performed before enrolment and used to guide physical examination.

A **general physical examination** is to be performed by a qualified health care professional and will include, at a minimum, a check of general appearance, auscultation of heart and lungs, palpation of the abdominal, assessment of extremities, and review of the subject's vital signs. "Qualified health care professional" refers to any licensed health care professional who is permitted by institutional policy to perform physical assessments and who is identified within the site's roles and responsibilities log. The vital signs may be measured by any health care professional allowed by institutional policy.

At clinic visits after enrollment, subjects will undergo a **symptom-directed physical examination** also to be performed by a qualified health care professional. This is a physical examination that will include a check of general appearance and examination of organ systems that are relevant to the investigator based on review of the subject's reported AEs and/or review of systems and/or concomitant medication use. Vital signs,

including body temperature (preferably oral), will also be measured and this may be performed by any professional allowed by institutional policy.

Corresponding information is documented in the source documents and relevant eCRFs.

6.3 Immunogenicity Measurements

- Immunogenicity will be determined by the HI assay against the A/H5N1 homologous strain.

Microneutralization (MN), as well as additional tests to further characterize the immune response might be performed for confirmatory purposes.

Immunogenicity will also be determined by HI assay for heterologous H5N1 strain(s), if tested.

Testing will be conducted by qualified and certified laboratories. For further details please see the Investigator Laboratory Manual which is located in the Investigator Site File.

HI antibody titers measured at Day 22 (21 days after first study vaccination), and at Day 43 (21 days after second study vaccination) are the adequate timings to measure the antibody response of subjects. These titers will be compared against baseline antibody titers (Day 1, prior to vaccination) and will be used to evaluate immunogenicity.

Additionally, measurement of HI antibody titers at Day 183 is appropriate to measure persistence of antibody titer. These titers will be compared against baseline and post-vaccination antibody titers and will be used to evaluate immunogenicity.

Please see [Section 7.5](#) for additional information regarding the assessment of immunogenicity parameters.

6.4 Efficacy Measurements

This study has no efficacy measurements.

6.5 Solicited Safety Measurements

The term “reactogenicity” refers to selected signs and symptoms (“AEs”) occurring in the hours and days following a vaccination, to be collected by the subject for 7 consecutive days, using a pre-defined check list in a diary card (i.e. solicited AEs), see [section 3.2.5.3](#) and [section 8.1](#).

The following AEs are included in the diary check list. Each AE is to be assessed using the scoring system shown in [Appendix B](#) and [Appendix C](#).

Solicited local adverse events:

Injection site induration, erythema, ecchymosis and pain.

Solicited systemic adverse events:

Nausea, generalized myalgia, generalized arthralgia, headache, fatigue, chills, loss of appetite, malaise and fever (derived from measured body temperatures (defined as body temperature ≥ 38.0 °C [≥ 100.4 °F])).

Other solicited adverse events:

Body temperature (summarized by route of measurement and in 0.5 °C increments from 36.0 °C) and the use of analgesics/ antipyretic medication for prophylaxis or treatment.

The study staff must review the diary card with the subject at the following visit (see [section 3.2.5](#)) and must directly record the solicited local and systemic AEs, and other solicited AEs on the appropriate Local and Systemic AEs eCRF. As described in [section 3.4.1](#), all solicited AEs that are legible must be recorded verbatim in the eCRFs, even if the values do not appear to be plausible.

If a solicited local or systemic AE continues beyond day 7 after vaccination, it will also be recorded as an AE on the AEs eCRF.

6.6 Unsolicited Safety Measurements

6.6.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

NOTE: Every effort should be made by the investigator to evaluate new safety information reported by a subject (solicited and unsolicited AEs) for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., “cough” or “ear pain”) are better reported according to the underlying cause (e.g., “asthma exacerbation” or “otitis media”).

All AEs will be monitored until resolution or, if the AE becomes chronic, a cause identified. If an AE is unresolved at the conclusion of the study, a clinical assessment will be made by the investigator and medical monitor whether continued follow-up of the AE is warranted.

The severity of events reported on the AEs eCRF will be determined by the investigator as:

Mild: transient with no limitation in normal daily activity.
Moderate: some limitation in normal daily activity.
Severe: unable to perform normal daily activity.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

1. Not Related

The AE is not related to an investigational vaccine if there is evidence that clearly indicates an alternative explanation. If the subject has not received the vaccine, the timing of the exposure to the vaccine and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that reasonably suggest an alternative explanation, then the AE is not related.

2. Possibly Related

The administration of the investigational vaccine and AE are considered reasonably related in time and the AE could be explained by exposure to the investigational vaccine or by other causes.

3. Probably Related

Exposure to the investigational vaccine and AE are reasonably related in time and no alternative explanation has been identified.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator. Solicited AEs will not be evaluated for relationship to study vaccine and severity of solicited AEs is defined as described in [section 6.5](#).

AEs will also be evaluated by the investigator for the co-existence of any of the other following conditions:

- “Medically attended AE”: an AE that leads to an unscheduled visit to a healthcare professional.

- “New onset of chronic disease”: an AE that represents a new diagnosis of a chronic medical condition that was not present or suspected in a subject prior to study enrollment.
- “AESIs”: Please see [Appendix A](#) for the full list of AESIs.

Please note: any solicited AE that meets any of the following criteria must also be entered as an AE on the AE eCRF:

- Solicited local or systemic AE leading to a “medically attended AE”.
- Solicited local or systemic AE leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator.
- Solicited local or systemic AE lasting beyond 7 days’ duration.
- Solicited local or systemic AEs that lead to subject withdrawal from study vaccination.
- Solicited local or systemic AE that otherwise meets the definition of a SAE (see [section 6.6.2](#)).

6.6.1.1 Adverse Events of Special Interest

Please see [Appendix A](#) for the full list of AESIs.

6.6.2 Serious Adverse Events

A SAE is defined as any untoward medical occurrence that at any dose results in one or more of the following:

- Death.
- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe.
- Required or prolonged hospitalization.
- Persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person’s ability to conduct normal life functions).
- Congenital anomaly/or birth defect.
- An important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

AEs which do not fall into these categories are defined as **non-serious**.

It should be noted that a severe AE need not be serious in nature and that a SAE need not, by definition, be severe.

SAEs will be captured both on the VSAE form as well as on the AE eCRF. All SAEs will be evaluated by the investigator for relationship of the event to study vaccine. SAEs that are judged to be possibly or probably related to the study vaccine should be reported to the Sponsor as related (i.e., suspected) events.

The relationship of the study treatment to an SAE will be determined by the investigator based on the following definitions:

1. Related/suspected

The SAE is judged by the investigator to be possibly or probably related to the study vaccine on the AE CRF page (see [section 6.6.1](#)).

2. Not Related

The SAE is not related if exposure to the study vaccine has not occurred, **or** the occurrence of the SAE is not reasonably related in time, **or** the SAE is considered unlikely to be related to use of the study vaccine, i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

In addition, SAEs will be evaluated by the Sponsor or designee for “expectedness.” An unexpected AE is one that is not listed in the current Summary of Product Characteristics or the Investigator’s Brochure or an event that is by nature more specific or more severe than a listed event.

In addition, a pre-existing event or condition that results in hospitalization should be recorded on the Medical History eCRF. If the onset of an event occurred before the subject entered the study (e.g., any pre-planned hospitalization for conditions like cosmetic treatments or for non-emergency routine visits for a pre-existing condition), the hospitalization would not lead to an AE being classified as serious unless, in the view of the investigator, hospitalization was prolonged as a result of participation in the clinical trial or was necessary due to a worsening of the pre-existing condition.

6.6.3 Methods for Assessing and Recording AEs and SAEs

The period of observation for AEs extends from the time the subject signs informed consent until he or she completes the specified safety follow-up period, approximately 13 months after the first vaccination or terminates the study early (whichever comes first).

AEs occurring after the ICF is signed but prior to receiving study vaccine/product will be documented as an AE and recorded on the AEs eCRF and within source documents. However, AEs occurring prior to receipt of any study vaccine will be analyzed separately from “treatment emergent” AEs (AEs occurring after administration of the first study vaccine).

All AEs meeting criteria for reporting, regardless of severity, will be monitored by the investigator until resolution or stabilization. All subjects experiencing AEs - whether considered associated with the use of the study vaccine or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist’s report should be supplied, if possible. All findings must be reported on an AEs eCRF and on the VSAE form, if necessary, which is part of the Investigator Site File. All findings in subjects experiencing AEs must be reported also in the subject's medical records.

All SAEs which occur during the course of the study, whether considered to be associated with the study vaccination or not, must be reported **within 24 hours of the site becoming aware of the event** by telephone or fax to the Sponsor. Contact details for submitting SAEs to the Sponsor or its designee and instructions for completion of documentation will be provided in a handout located in the Investigator Site File.

All SAEs are also to be documented on the AE eCRF. Any medication or other therapeutic measures used to treat the AE will be recorded on the appropriate eCRF(s) in addition to the outcome of the AE.

After receipt of the initial report, representatives of the Sponsor will contact the investigator if it is necessary to obtain further information for assessment of the event.

All SAEs must be reported by the investigator to his/her corresponding EC or IRB and applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the Sponsor.

The Sponsor or its designee must also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious and non-serious adverse vaccine reactions (also referred to as “SUSARs”) to the regulatory authority(ies) and the IRB/EC. If a SUSAR or other safety signal relating to use of one of the study vaccines is reported to the Sponsor or its designee, the Sponsor will communicate the information to the investigator and the investigator will be responsible for submitting this information to the EC or IRB and other relevant authorities.

Post-Study Events

Any suspected SAE that occurs outside of the protocol-specified follow-up period or after the end of the study but considered to be caused by the study vaccine must be reported to the Sponsor or its designee. Instructions and contact details for collecting and reporting these suspected SAEs will be provided to the investigator.

6.6.4 Pregnancies

To ensure subjects' safety, each pregnancy in a subject on study vaccine must be reported to the Sponsor within 24 hours of the site learning of its occurrence. If the subject agrees to submit this information, the pregnancy must be followed to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if intended duration of safety follow-up for the study has ended.

Pregnancy data must be recorded on a Pregnancy Report CRF (initial report) and Pregnancy Follow-Up CRF (outcome report) and reported to the Sponsor. Contact details for submitting the case report forms will be described in the Investigator Site File.

Any pregnancy outcome meeting the definition of a SAE (see [section 6.6.2](#)) must also be reported on the VSAE Report Form.

6.7 Safety Laboratory Measurements

This study has no safety laboratory measurements.

6.8 Other Measurements

This study has no other measurements.

6.9 Data Monitoring Committee

No Data Monitoring Committee is planned in this study.

7.0 ENDPOINTS AND STATISTICAL ANALYSES

7.1 Endpoints

7.1.1 Primary Immunogenicity Endpoints

The primary immunogenicity endpoints will be based on HI antibody response to the H5N1 vaccine strain for subjects ≥ 18 years of age, in the aH5N1c vaccine groups only.

- GMT at Day 43 by lot
- Percentage of subjects with HI titer $\geq 1:40$ on Day 43 by age cohort (18 to <65 years of age and ≥ 65 years of age) for the pooled lots.

7.1.2 Secondary Immunogenicity Endpoints

The secondary measures of immunogenicity, as determined by the HI assay, against the H5N1 homologous strain, include the following:

- GMT at Day 1, Day 22, Day 43, and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥ 65 years of age).
- Percentage of subjects with HI titer $\geq 1:40$ on Day 1, Day 22, and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥ 65 years of age).
- Percentage of subjects achieving seroconversion (defined as: HI titer $\geq 1:40$ for subjects negative at baseline [HI titer $< 1:10$]; or a minimum 4-fold increase in HI titer for subjects positive at baseline [HI titer $\geq 1:10$]) on Day 22, and Day 43 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <65 years of age and ≥ 65 years of age).
- Geometric mean HI titer (GMT) at Day 1, Day 22, Day 43 and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <60 years of age and ≥ 60 years of age)
- Percentage of subjects with HI titer $\geq 1:40$ on Day 1, Day 22, Day 43, and Day 183 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <60 years of age and ≥ 60 years of age)
- Percentage of subjects achieving seroconversion (defined as: HI titer $\geq 1:40$ for subjects negative at baseline [HI titer $< 1:10$]; or a minimum 4-fold increase in HI titer for subjects positive at baseline [HI titer $\geq 1:10$]) on Day 22, and Day 43 by vaccine

group (aH5N1c or placebo) and by age cohort (18 to <60 years of age and \geq 60 years of age)

- Geometric mean ratio (GMR) of HI titer: Day 22/Day 1, Day 43/Day 1 by vaccine group (aH5N1c or placebo) and by age cohort (18 to <60 years of age and \geq 60 years of age)

7.1.3 Secondary Efficacy Endpoints

Not applicable.

7.1.4 Safety Endpoints

The measures for assessing safety and tolerability are as follows:

- Percentages of subjects with solicited local, solicited systemic and other AEs as measured for 7 days (inclusive) following each (1st and 2nd) and any (1st or 2nd) vaccination, by vaccine group (and calculated for several time intervals after vaccination⁷: 30 minutes, 1 to 3 days (without 30 min), 4 to 7 days, and 1 to 7 days (without 30 min), and 1 to 3 days (including 30 minutes) and 1 to 7 days (including 30 minutes).
- Percentages of subjects with any unsolicited AEs reported through 21 days after each (1st and 2nd) and any (1st or 2nd) vaccination by vaccine group.
- Percentages of subjects reporting SAEs, AESIs, NOCD, AEs leading to vaccine/study withdrawal, and medically attended AEs, and concomitant medications associated with these events as collected from Day 1 to Day 387, by vaccine group.

7.1.5 Other Endpoints

Not applicable.

7.1.6 Exploratory Endpoints

Not applicable.

⁷ Timeframes shown relate to each vaccination; for second vaccination, please see Time and Events table for corresponding study visit days for each procedure

7.2 Success Criteria

7.2.1 Success Criteria for Co-Primary Objectives

Lot-to-lot consistency, derived from Day 43 HI titers for each aH5N1c lot, will be claimed if, for subjects ≥ 18 years of age in the Per Protocol Set, the two-sided 95% confidence intervals (CIs) of all the three pairwise comparisons (GMT(Group A)/ GMT(Group B), GMT(Group A)/ GMT(Group C), GMT(Group C)/ GMT(Group B)) fall within 0.667 and 1.5. Significance level to all these tests is $\alpha = 5\%$, which needs no adjustment for multiplicity as all hypotheses have to be rejected (intersection-union test problem).

Furthermore after lot-to-lot consistency is achieved, CBER criteria for the percentage of subjects with HI titer $\geq 1:40$ on Day 43 will be assessed as below, for subjects from both age cohorts (18 to 65 years of age and ≥ 65 years of age) on pooled lots. The overall power to fulfill those CBER criteria is 98%.

Immunogenicity according to CBER in subjects 18 - < 65 years of age

The aH5N1c vaccines immunogenicity will be evaluated in subjects 18 to < 65 years of age, using the following measurements according to the current CBER criteria for the adult population (CBER 2007):

- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 70%.

Immunogenicity according to CBER in subjects ≥ 65 years of age

The aH5N1c vaccine immunogenicity will be evaluated in subjects ≥ 65 years of age, using the following measurements according to the current CBER criteria for the ≥ 65 years of age population (CBER 2007):

- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 60%.

The study will be considered a success if both lot-to-lot consistency for subjects aged ≥ 18 years, and CBER criteria (titer above 1:40) for both age cohorts (18-65 years and ≥ 65 years) are met.

7.2.2 Success Criteria for Secondary Immunogenicity Objectives

CBER criteria for seroconversion at Day 43 will be evaluated for both age cohorts as defined below. Results collected at Day 22 will also be evaluated against the CBER criteria for both age cohorts.

Immunogenicity according to CBER in subjects 18 - < 65 years of age

The aH5N1c vaccines immunogenicity will be evaluated in subjects 18 to < 65 years of age, using the following measurements according to the current CBER criteria for the adult population (CBER 2007):

- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 70%.
- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40%.

Immunogenicity according to CBER in subjects ≥ 65 years of age

The aH5N1c vaccine immunogenicity will be evaluated in subjects ≥ 65 years of age, using the following measurements according to the current CBER criteria for the ≥ 65 years of age population (CBER 2007):

- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 60%.
- The lower bound of the adjusted two-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 30%.

To assess CHMP criteria (CPMP/BWP/214/96), the estimate of proportions of subjects with HI titer $\geq 1:40$ and proportions of subjects achieving seroconversion and GMR will be compared against threshold as follows:

Criteria for subjects 18 to 60 years of age:

- The percentage of subjects with seroconversion in HI antibody is $>40\%$;
- The GMR is >2.5 ;
- The percentage of subjects achieving an HI titer $\geq 1:40$ is $>70\%$.

For subjects > 60 years of age:

- The percentage of subjects with seroconversion in HI antibody is $>30\%$;
- The GMR is >2.0 ;
- The percentage of subjects achieving an HI titer $\geq 1:40$ is $>60\%$.

7.2.3 Success Criteria for Secondary Efficacy Objectives

Not applicable.

7.2.4 Success Criteria for Safety Objectives

Not applicable.

7.3 Analysis Sets

7.3.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments, regardless of the subject's randomization and treatment status in the study.

7.3.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3.3 Full Analysis Set (FAS) Immunogenicity Set

All subjects in the All Enrolled Set who:

- actually receive at least one dose of study vaccination, and
- provide at least one evaluable serum sample at relevant timepoints.

FAS will be defined by timepoint and objective, for example:

- FAS1 (applicable to lot-to-lot objective, GMT at Day 43, the percentage of Subjects achieving an HI antibody titer $\geq 1:40$ at Day 43) will include all subjects who received at least one dose of study vaccine and provide at least one evaluable serum sample at Day 43.
- FAS2 (applicable to CBER criterion on seroconversion or significant increase) will include all subjects who received at least one dose of study vaccine and provide at least one evaluable serum sample at Day 1 AND Day 43.

More details will be provided in the statistical analysis plan.

FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

7.3.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity Set who:

- Are not excluded due to reasons (see [section 7.3.8](#)) defined prior to unblinding or analysis.

Examples for subjects excluded due to other reasons than major protocol deviations are:

- subjects who withdrew informed consent.

Similarly to FAS, PPS will also be defined per objective and timepoint.

Exclusions will be considered by objective/time point, i.e., sometimes not all data of a subject but only part of the subject's data will be removed from the PPS analysis.

7.3.5 Safety Set

Safety Set (solicited adverse events and other solicited adverse events)

All subjects in the Exposed Set who:

- Provide post vaccination solicited AE data.

Safety Set (unsolicited adverse events)

All subjects in the Exposed Set who:

- Have post-vaccination unsolicited AE records.

Safety Set (overall)

All subjects in the Exposed Set who:

- Have either post-vaccination solicited or unsolicited AE records.

Subjects will be analyzed as "treated" (i.e., according to the vaccine a subject received, rather than the vaccine to which the subject may have been randomized).

7.3.6 Other Analysis Sets

Not applicable.

7.3.7 Subgroups

Subgroup analyses also will be performed by stratifying the study population as appropriate for immunogenicity or safety parameters, as further detailed in the Statistical Analysis Plan (AP):

- Subjects stratified by baseline HI titer $<1:10$ or $\geq 1:10$
- Subjects with and without seasonal influenza vaccine in the last 12 months
- Subjects by age (CBER and CHMP defined)
- Subjects stratified by age: 18-49, 50-64, 65-74, over 75
- Subjects by race

- Subjects by sex
- U.S. subjects and non U.S. subjects

7.3.8 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. A protocol deviation may be a reason to remove data from an analysis set at the time of analysis. Reportable (major) protocol deviations will be defined as exclusionary from the analysis according to protocol objectives and endpoints, which will be specified in the statistical analysis plan. In some cases exclusion of data may be due to a reason other than a protocol deviation, e.g. early termination.

7.4 Analysis Plan

7.4.1 Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrollment will be calculated by overall and by vaccine group.

Distributions of subjects by sex, ethnicity, race and previous influenza vaccination (in the past 12 months) will be summarized overall and by vaccination group.

7.4.2 Analysis of Co-Primary Objectives

For lot-to-lot consistency, the following equivalence hypotheses will be tested simultaneously:

$$H_0: (\mu_{\text{lot A}} - \mu_{\text{lot B}}) \leq -0.176 \text{ or } (\mu_{\text{lot A}} - \mu_{\text{lot B}}) \geq 0.176 \text{ or} \\ (\mu_{\text{lot A}} - \mu_{\text{lot C}}) \leq -0.176 \text{ or } (\mu_{\text{lot A}} - \mu_{\text{lot C}}) \geq 0.176 \text{ or} \\ (\mu_{\text{lot B}} - \mu_{\text{lot C}}) \leq -0.176 \text{ or } (\mu_{\text{lot B}} - \mu_{\text{lot C}}) \geq 0.176$$

vs.

$$H_1: (\mu_{\text{lot A}} - \mu_{\text{lot B}}) > -0.176 \text{ and } (\mu_{\text{lot A}} - \mu_{\text{lot B}}) < 0.176 \text{ and} \\ (\mu_{\text{lot A}} - \mu_{\text{lot C}}) > -0.176 \text{ and } (\mu_{\text{lot A}} - \mu_{\text{lot C}}) < 0.176 \text{ and} \\ (\mu_{\text{lot B}} - \mu_{\text{lot C}}) > -0.176 \text{ and } (\mu_{\text{lot B}} - \mu_{\text{lot C}}) < 0.176$$

Here, H_1 refers to the alternative hypothesis of pairwise equivalence (consistency) transformed to the log₁₀ scale. Accordingly, $\mu_{\text{lot A}}$, $\mu_{\text{lot B}}$, and $\mu_{\text{lot C}}$ denote the means of log₁₀-transformed Day 43 titers of the corresponding lot groups. The lot-to-lot consistency will be claimed if the two-sided 95% CIs of all the three pairwise

comparisons are within the equivalence ranges. Significance level to all these tests is $\alpha = 5\%$, which needs no adjustment for multiplicity as all hypotheses have to be rejected (intersection-union test problem).

For CBER criteria HI antibody titer $\geq 1:40$ should meet or exceed 70% and 60% respectively for the two different age cohorts (18- <65 years of age and ≥ 65 years of age) the following hypothesis will be tested:

$$H_0: (\pi_i - \pi_0) \leq 0 \quad \text{vs.} \quad H_1: (\pi_i - \pi_0) > 0$$

Here, H_1 refers to the alternative hypothesis. π_0 denotes the threshold for proportion of subjects with a HI titer $\geq 1:40$ ($\pi_0=0.7$ for subjects 18 to <65 years of age and $\pi_0=0.6$ for subjects ≥ 65 years of age). Significance levels to these tests is $\alpha = 5\%$ which needs no adjustment for multiplicity as all hypothesis have to be rejected (intersection union problem).

7.4.2.1 Analysis Populations for Co-Primary Objectives

The primary analysis population for testing the null hypotheses above will be the Per Protocol Set (PPS).

7.4.2.2 Statistical Methods for Co-Primary Objectives

Log-normal distributed data

All statistical analyses for HI will be performed on the logarithmically (base 10) transformed values. Individual HI titers below detection limit will be set to half that limit.

Geometric mean HI antibody titers (GMT)

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% CIs at Day 43 will be determined using analysis of covariance (ANCOVA) with factors for lot, age groups (18-64, ≥ 65), center and a covariate for the effect defined by the log-transformed prevaccination antibody titer. Influenza-antibody GMTs (Day 43), associated two-sided 95% CIs and median, minimal, and maximal area/titer values will be determined and presented together with N (number of subjects), by lot (for the first primary objective) . This model will include only immunogenic data from the 3 lots of aH5N1.

Statistical analyses will be performed on the logarithmically (base 10) transformed titer values, while median, minimum, and maximum values will be obtained on the actual titer values.

To assess equivalence of lots, the limit of the 95% CI will be compared with the pre-specified thresholds (see [Section 7.2.1](#)).

Analysis of binary data

Binary data (proportion of subjects with HI titer $\geq 1:40$) will be analyzed using loglinear models with a factor for dose group adjusted for center, at Day 43. Proportions and two-sided 95% CIs will be calculated based on these models. Models might be simplified in case of convergence problems.

Handling of missing values for Immunogenicity Data

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), i.e., not informative. Therefore, the primary analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

7.4.2.3 Sample Size and Power Considerations of Co-Primary Objectives

Data observed in previous studies in similar sets (V89_04 in subjects 18-64 years of age; V89_13 in subjects > 65 years of age) showed a variability of the post vaccination titers ranging from 0.75 to 0.88 (in the log scale). With the proposed sample size, assuming a standard deviation of 0.85 for the log₁₀ antibody titers (for each vaccine lot), approximate pairwise equivalence of factor 1 and independency, a single equivalence test based on 718 subjects per lot group has a power of 95%. The resulting overall power is approximately 86%, because the total number of comparisons is three. To account for dropouts (approximately 10%), a total of $n=798$ per lot should be recruited.

Data observed in previous studies in similar populations (V89_04 in subjects 18- <65 years of age; V89_13 in subjects > 65 years of age) showed a proportion of 86% of subjects aged 18 to <65 years and a proportion of 81% of subjects aged 65 years and older achieving an HI antibody titer of $\geq 1:40$. With a sample size of 1077 evaluable subjects from the pooled lots there is an overall power of approximately 98% to achieve the CBER criteria (HI antibody titer $\geq 1:40$ for at least 70% of the subjects aged 18 to <65 years, and for at least 60% of the subjects aged 65 years and older). To account for dropouts (approximately 10%), a minimum sample size of $n=1197$ for all lots combined are needed.

7.4.3 Analysis of Safety Objectives

Handling of missing values for Safety Data:

Unsolicited AEs: The entire study period will be divided into disjoint intervals based upon the time and event schedule in the protocol. Endpoints for the intervals will be specified in the AP.

Solicited AEs: The solicited study period (30min-Day 7) will be divided into disjoint intervals: 30 min, 6h-Day 3, Day 4-Day 7, and one overall interval: 6h-Day 7.

For each of the intervals the following algorithm will be applied:

1. If less than 20% of subjects are without any solicited AE data (i.e., none of the solicited AEs has been captured in the interval) for the respective time interval, then no further action is necessary and the Safety Set pertaining to the interval will be analyzed.
2. If 20% or more of subjects are without any solicited AE data, the missing mechanism will be analyzed by vaccine group using a newly created variable indicating whether a subject is missing the respective AE-value or not (1=AE record present; 0=AE record not present).
 - a. If the percentage of missing subjects does not vary significantly between vaccine groups ($p > 0.05$) then 'missing completely at random' (MCAR) is assumed and no further action is required. Data will be analysed without the missing values.
 - b. If the percentage of missing subjects vary significantly between vaccine groups ($p \leq 0.05$) then 'missing at random' (MAR) is assumed, i.e., the missing mechanism is conditional on the vaccine group. Multiple imputation methods will be applied to reduce potential bias arising from missing values (details will be given in Appendix 16.1.9 of the CSR, if applicable).

For solicited events, multiple imputation will be confined to analyses of any (rather than specific) local AEs and of any (rather than specific) system reactions and for unsolicited events, to analyses of any AEs and SAEs.

Further details of the statistical methods will be provided in the AP.

7.4.3.1 Analysis of Extent of Exposure

The number of subjects actually receiving the first and the second vaccination will be summarized by vaccine group.

7.4.3.2 Analysis of Solicited Local and Systemic Adverse Events and Other Adverse Events

There is no statistical null hypothesis associated with the safety objective, which will be analyzed descriptively. All safety analyses will be done by vaccine group. Safety will also be analyzed by age.

All solicited AEs will be summarized according to defined severity grading scales ([Appendices A and B](#)).

Frequencies and percentages of subjects experiencing each AE will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic AE overall and at each time point will also be presented.

Post-vaccination solicited AEs reported from Day 1 to Day 7 will be summarized for the intervals Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval), by maximal severity and by vaccine group. The 30-minute interval measurement will be summarized separately. The severity of solicited local AEs, including injection-site erythema, ecchymosis and induration will be categorized by grades based on the linear measurement of these AEs. Injection-site erythema, ecchymosis and induration will be summarized according to categories based on linear measurement: mild (grade I): 2.5 cm to 5.0 cm; moderate (grade II): 5.1cm to 10 cm;severe (grade III): 10 cm.

Injection site pain and systemic AEs (except fever) occurring for the 7 days including each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic AE will also be further summarized as “none” versus “any”.

Use of antipyretics and analgesics will be summarized by frequency and percentage of subjects reporting use and by whether or not these medications were used for treatment and/or prophylaxis. The influence of antipyretics and analgesics use on the occurrence of specific AEs (e.g., fever) will be assessed.

Body temperature will be summarized by 0.5 ° C increments from <36.0° C up to ≥40.0° C and will be broken down irrespective of route of measurement. Body temperature will also be summarized as <38.0 °C or ≥38.0 °C.

Biologically implausible measurements (for further definition see analysis plan) will be left out of the analyses of these solicited events.

7.4.3.3 Analysis of Spontaneously Reported Adverse Events

All the AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded.

The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The AEs will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group and by interval of study observation. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- SAEs
- AEs that are possibly or probably related to vaccine
- AEs of special interest
- new onset of chronic disease
- AEs leading to vaccine/study withdrawal
- AEs leading to a medically attended visit
- AEs by data source

Data listings of all AEs will be provided by subject. In addition, AEs in the categories above will be provided as listed data.

7.4.3.4 Analysis of Safety Laboratory Values

Not applicable.

7.4.4 Analysis of Secondary Immunogenicity Objectives

Log-normal distributed data

All statistical analyses for HI will be performed on the logarithmically (base 10) transformed values. Individual HI titers below detection limit will be set to half that limit.

Geometric mean HI antibody titers (GMT)

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% CIs at Days 1, 22, 43 and 183 will be determined using analysis of covariance (ANCOVA) with factors for vaccine dose group and center, by age cohort. GMTs at Days 22, 43 and 183 will be adjusted by the pre-vaccination antibody titer. Influenza-antibody GMTs, associated two-sided 95% CIs and median, minimal, and maximal area/titer values will be determined and presented together with N (number of subjects), by vaccine group in both age cohorts (for CBER and CHMP objectives). Statistical analyses will be performed on the logarithmically (base 10) transformed titer values, while median, minimum, and maximum values will be obtained on the actual titer values.

Analysis of binary data

Binary data (proportion of subjects with HI titer $\geq 1:40$ and proportions of subjects achieving seroconversion or significant increase) will be analyzed using loglinear models with a factor for dose group adjusted for center, at Days 1, 22, 43 and 183. Proportions and two-sided 95% CIs will be calculated based on these models. Models might be simplified in case of convergence problems.

To assess CBER criteria, the lower limit of the 95% CI of the two endpoints (proportion of Subjects with HI titer $\geq 1:40$ and proportions of subjects achieving seroconversion or significant increase) will be compared with the pre-specified thresholds (see [Section 7.2.1](#)), in the two age cohorts (18-64, ≥ 65).

In addition, results collected at Day 22 will also be evaluated against the CBER criteria for both age cohorts.

Results collected at Day 22 will also be evaluated against the CHMP criteria.

The analysis population for immunogenicity analysis regarding CBER and CHMP will be based on the Full Analysis Set (FAS).

Handling of missing values for Immunogenicity Data:

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), i.e., not informative. Therefore, the primary analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

7.5 Planned Analyses

A formal interim analysis of immunogenicity will be carried out by an independent statistician and programmer (to preserve the blind) after Day 43 immunogenicity data are available for all subjects (excluding those lost to follow-up or withdrawn for other reasons). No adjustments for multiplicity are needed for the interim analysis. Antibody persistence immunogenicity data (Day 183) and safety data (to Day 387) will be included in a final analysis and presented in the CSR. Further details are given in the SAP.

The final analysis will be performed when all data up to the study end (Day 387) are available. Analysis will be done on fully unblinded data by the Sponsor and all data will be presented in the final, cumulative study report.

8.0 SOURCE DOCUMENTATION, Study Monitoring, AND Auditing

In order to ensure consistency across sites, monitoring and auditing will be standardized and performed in accordance with the Sponsor's or delegated contract research organization's (CRO) standard operating procedures and applicable regulatory requirements (e.g., FDA, EMA, and ICH guidelines).

Prior to enrollment of the first study subject, the Sponsor or delegate will train investigators and/or their study staff on the study protocol, all applicable study procedures, documentation practices (including signing of the source data agreement (SDA, see [section 8.1](#)) and all electronic systems. CRFs supplied by the Sponsor must be completed for each enrolled subject (see [section 7.3.1](#) for definition of enrolled subject). Documentation of screened but not enrolled subjects must be maintained at the site and made available for review by the site monitor. All data entries as well as study related documents will be checked by the Sponsor and/or site monitor. In addition, the investigator and site staff will be made aware of the plans to monitor the data collected at the site.

8.1 Source Documentation

Prior to the start of the study, the site staff participating in the study conduct will be trained on what documents will be required for review as source documentation (i.e., original records, laboratory reports, medical records, subject diary cards). The kinds of documents that will serve as source documents will be specified in the SDA. The SDA will be finalized and available for further review prior to first subject, first visit.

In addition, source documentation **must** include all of the following: subject identification (on each page), eligibility and participation, proper informed consent procedures, dates of visits, adherence to protocol procedures, adequate reporting and follow-up of AEs, documentation of prior/concomitant medication/vaccines, study vaccine receipt/dispensing/return records, study vaccine administration information, any data collected by a telephone conversation with the subject and date of completion and reason.

The subject must also allow access to their medical records. Each subject must be informed of this prior to the start of the study and consent for access to medical records may be required in accordance with local regulations.

All safety data reported by subjects must be written down in source documents prior to entry of the data into eCRFs. If there are multiple sources of information (e.g., diary card, verbal report of the subject, telephone contact details, medical chart) supporting the diagnosis of an AE, these sources must be identified in the source documents, discrepancies between sources clarified, the ultimate diagnosis must be justified and

written in the source documents, and this diagnosis must be captured in the AE eCRF . The AE CRF must also capture which source(s) of information were used to determine the AE (e.g., subject recall, medical chart, diary card, and/or other sources).

8.2 Study Monitoring and Source Data Verification

A contract research organization (CRO) may be involved in the monitoring of protocol conduct and data entry. If a CRO is involved in study oversight, the name and address of this CRO will be located in the investigator site file. Prior to enrollment of the first study subject, the CRO will develop a Clinical Monitoring Plan to specify how monitoring will be performed for the study.

Study progress will be monitored by the Sponsor or its representative (e.g. a CRO) as frequently as necessary to ensure:

- that the rights and well-being of human subjects are protected
- the reported trial data are accurate, complete, and verifiable from the source documents and
- the conduct of the trial is in compliance with the current approved protocol/amendment(s), GC and applicable regulatory requirements

Contact details for the team involved in study monitoring will be identified in a handout located in the Investigator Site File. Study data recorded on eCRF/CRFs will be verified by checking the eCRF/CRF entries against source documents in order to ensure data completeness and accuracy as required by study protocol. Additional documents such as the investigator site file, pharmacy records, and informed consent documentation must also be available for review if requested. Arrangements for monitoring visits will be made in advance in accordance with the monitoring plan, except in case of emergency.

The investigator and/or site staff must make source documents of subjects enrolled in this study available for inspection by the Sponsor or its representative at the time of each monitoring visit. These documents must also be available for inspection, verification and copying, as required by regulations, by officials of the regulatory health authorities (e.g., FDA, EMA and others) and/or ECs/IRBs. The investigator and study site staff must comply with applicable privacy, data protection and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

9.0 DATA MANAGEMENT

9.1 Data Entry and Management

In this study, data will be entered onto eCRFs in a timely fashion by the investigator and/or the investigator's dedicated site staff. Data entered onto eCRFs are stored on a secure website. The data collected on this secure website are assimilated into an EDC system, which is compliant with 21 Part 11 policies of the Code of Federal Regulations. The EDC will be designed and validated by the Sponsor or its representative(s) prior to activation for data entry by sites. The investigator must review data entered and electronically sign the eCRFs to verify their accuracy.

Access to the EDC system for data entry or review will require training and distinct individual access code assignments to those site staff members who will be entering study data and those involved in study oversight who may review study data. Data are collected within EDC, to which the Sponsor and site monitors have exclusively "read only" access. eCRF data will be reviewed routinely by study personnel from the Sponsor or its representative(s) and clinical monitors.

Pregnancy CRFs that are three-part "no carbon required" (NCR) paper CRFs will be provided for each subject by the Sponsor. One copy must be retained by the investigator, and all other copies (including the original copy) will be returned as directed by the Sponsor. Instructions on how to complete these forms will be provided to the investigator.

All study data must be entered by the investigator or delegate who will sign and date the CRFs. If the investigator delegates and authorizes other persons in his/her staff to make entries on the CRF, the names, positions, signatures and initials must be documented in writing (e.g., site delegation log).

Arrangements will be made by the study monitor to collect the CRFs upon completion. No CRFs are to be mailed to the Sponsor without specific authorization.

Data from paper CRFs are entered into the study database by Sponsor staff or delegates using single data entry. Verification is performed manually by a separate member of the staff by comparing the CRF to the data entered into the database.

Electronic Data Transfer (EDT) is one method used for collecting laboratory data. The full-service laboratory (i.e., central laboratory) will send data as electronic files by a secured method (e.g. via diskette, CD, as an encrypted file attachment on electronic mail, or as a direct transfer into a specified server directory) to the Sponsor's CDMS department. The data file is pre-processed and loaded by a member of the CDMS team

into the study database. The laboratory will submit a results file containing the tests and the results as specified in the protocol. If the laboratory provides the service, it will also submit a Demography (DEMOG) file containing the subject's demographic information. If the file includes results of data blinded to personnel in clinical research, the source will provide a separate results file that will be loaded into a separate laboratory table.

For this study, antibody laboratory data may be transmitted via EDT.

9.2 Data Clarification

As part of the conduct of the trial, the Sponsor may have questions about the data entered by the site, referred to as queries. The monitors and the Sponsor are the only parties that can generate a query.

For eCRF trials, all corrections and clarifications will be entered into the EDC and will be identified by the person entering the information, the reason for the change, as well as the time of the changes made. If changes are made to a previously and electronically signed CRF, the investigator must confirm and endorse the changes.

9.3 Data Coding Procedures

Coding of AEs, Medical History, and Prior and Concomitant Medications will be performed using standard dictionaries.

9.4 Data Protection

The Sponsor respects the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations.

10.0 RECORD RETENTION

Investigators must retain all study records required by the Sponsor and by the applicable regulations in a secure and safe facility. The investigator must consult a Sponsor representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition, or custody of the study files. Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. The Committee for Human Medicinal Products for Human Use (CHMP) requires retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6, 4.9.5). It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained (ICH E6, 5.5.12).

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

11.0 USE OF INFORMATION AND PUBLICATION

The Sponsor assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov, and in compliance with current regulations.

The Sponsor also assures that key results of this clinical trial will be posted in a publicly accessible database within the required time-frame from the last subject's last study visit as dictated by applicable regulations.

Further to legislated data disclosure, the Sponsor will ensure that as far as possible results of this study will be published as scientific/clinical papers in high-quality peer-reviewed journals. Preparation of such manuscripts will be made with full collaboration of principal investigators and in accordance with the current guidelines of Good Publication Practice ([Graf 2009](#)).

The Sponsor must be notified of any intent to publish data collected from the study and prior approval from the Sponsor must be obtained prior to publication.

12.0 ETHICS

12.1 Regulatory and Ethical Compliance

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations including the US Code of Federal Regulations Title 21, the Sponsor's codes on protection of human rights, and with the ethical principles laid down in the Declaration of Helsinki (European Council 2001, US Code of Federal Regulations, ICH 1997).

12.2 Informed Consent Procedures

Eligible subjects may only be included in the study after providing written informed consent, as described in [section 3.2.1](#). Before the start of the trial, the investigator will have the informed consent and any other materials that will be provided to the subjects reviewed and approved by the IRB/EC. This review and approval will be documented and stored with other study documents. The investigator or designee must fully inform the subject of all pertinent aspects of the trial. A copy of the written informed consent will be given to the subject or the designee. The subject/designee must be allowed ample time to ask about the details of the trial and to make a decision as to whether or not to participate in the study. The subject must sign the consent form indicating their agreement to participate in the study before any study-related procedures are conducted. If the subject is unable to read and write, a witness must be present during the informed consent discussion and at the time of informed consent signature.

Prior to the start of the study, the Sponsor will provide to investigators a separate document with a proposed ICF that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by the Sponsor before submission to the IRB/EC and a copy of the approved version must be provided to the Sponsor's monitor after IRB/EC approval.

Women of child bearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirements indicated in the protocol. If case of doubts on the ability of a subject to adhere to these requirements, that subject should not be allowed in the study

12.3 Responsibilities of the Investigator and IRB/EC

The protocol and the proposed ICF must be reviewed and approved by a properly constituted IRB/EC before study start. Properly constituted IRB/EC is defined in ICH Guideline for Good Clinical Practice E6 (R1), Section 3 (ICH 1997). A signed and dated statement that the protocol and informed consent have been approved by the IRB/EC must be given to the Sponsor before study initiation. Prior to study start and at any time the protocol is amended during study conduct, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to the Sponsor's monitors, auditors, the Sponsor's Clinical Quality Assurance representatives, designated agents of the Sponsor, IRBs/ECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform the Sponsor immediately that this request has been made.

The investigator is also responsible for the following:

- maintaining a list of appropriately qualified persons to whom the investigator has delegated significant trial-related duties
- demonstrating the capability of recruiting the required number of suitable subjects within the recruitment period
- demonstrating sufficient time and staffing to properly conduct and complete the study within the agreed trial period
- ensuring that all persons assisting with the study are adequately informed about the protocol, the investigational product(s), and their study-related duties and functions
- ensuring that appropriately trained health care professionals are responsible for all study-related medical decisions and for ensuring appropriate medical care of subjects experiencing any AE related to the study
- if permission to do so is given by the subject, ensuring that the subject's primary healthcare provider is informed of the subject's participation in the study.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor(s), change of telephone number(s)). In addition, the investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

- (a) to the IRB/IEC for review and approval/favourable opinion,
- (b) to the Sponsor for agreement and, if required,
- (c) to the regulatory authority(ies).

12.4 Protocol Adherence

Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact the Sponsor or its agents, if any, monitoring the trial to request approval of a protocol deviation, as no authorized deviations are permitted. If the investigator feels a change to the protocol would improve the conduct of the trial this must be considered a protocol amendment, and unless such an amendment is agreed upon by the Sponsor and approved by the IRB/EC it cannot be implemented. All significant protocol deviations will be recorded and reported in the Clinical Study Report.

12.5 Protocol Amendments

An amendment is a written description of change(s) to or formal clarification of a trial protocol which may impact on the conduct of the clinical trial, potential benefit of the clinical trial, or may affect subject safety, including changes of trial objectives, trial design, subject population, sample sizes, trial procedures, or significant administrative aspects. An administrative change of a trial protocol is a minor correction or clarification that has no significant impact on the way the clinical trial is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes). Protocol amendments must be approved by the Sponsor, Health Authorities where required, and the IRB/EC. In cases when the amendment is required in order to protect the subject safety, the amendment can be implemented prior to IRB/EC approval. Notwithstanding the need for formal approval of a protocol amendment, the investigator is expected to take any immediate action required for the safety of any subject included in this trial, even if this action represents a deviation from the protocol. In such cases, the Sponsor should be notified of this action, the IRB/EC at the trial site, and, if required by local regulations, the relevant health authority) should be informed within 10 working days.

13.0 REFERENCE LIST

Committee for proprietary medicinal products (CPMP), European Agency for the Evaluation of Medicinal Products. (2003) Guideline on dossier structure and content for pandemic influenza vaccine marketing authorization application (Revision). London UK

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Committee for proprietary medicinal products (CPMP), European Agency for the Evaluation of Medicinal Products. (2007) Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context. London, UK

Fedson DS (2003) Pandemic Influenza and the Global Vaccine Supply. CID; 36: 1552 - 1561

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Graf C, Battisti WP, Bridges D, et al. (2009) Good publication practice for communicating company sponsored medical research: the GPP@ guidelines. BMJ 339:b4330

Nicholson KG, Colegate AE, Podda A, et al. (2001) Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. The Lancet; 357: 1937 - 1943

Treanor J (2004) Influenza Vaccine – Outmaneuvering Antigenic Shift and Drift. N Engl J Med; 350: 218 - 220 1.

Treanor J, Campbell JD, Zangwill KM, Rowe T, Wolff M (2006) Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med; 354: 1343 – 1351

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): Guidance for Industry Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): Guidance for Industry; Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

World Health Organization: Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO. Available at:
http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html. Accessed 7 October 2013

59th World Medical Association General Assembly (October 2008) Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Seoul, Korea

APPENDIX A: ADVERSE EVENTS OF SPECIAL INTEREST

AEs of special interest (AESI) will include those listed in below. The AESIs will be defined according to the following MedDRA preferred terms.

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

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: TOXICITY GRADING SCALES FOR LOCAL ADVERSE EVENTS

(Adapted from [CBER 2007b](#)*)

Adverse event Following Administration of Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Erythema/ Induration/ Swelling / Ecchymosis ⁸	2.5 – 5 cm	5.1 – 10 cm	> 10 cm

*This toxicity grading scale is adapted from CBER 2007b to enable ease of reporting by subjects in the diary cards for ‘patient reported’ solicited adverse events. This toxicity grading scale is a Sponsor standard that is used throughout the Influenza clinical programs for patient reporting. ‘Grade 4’ is not listed here but will be defined in the statistical analysis plan as necessary.

⁸ These ranges will be included in analyses, summarized: 1 to 2.4 cm, 2.5 to 5.0 cm, 5.1 to 10.0 cm, > 10.0 cm.

APPENDIX C: TOXICITY SCALES FOR SYSTEMIC ADVERSE EVENTS

(Adapted from [CBER 2007b](#)*)

Systemic Adverse event		Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever	°C	38.0 – 38.4	38.5 – 38.9	39.0 – 40
	°F	100.4 – 101.1	101.2 - 102	102.1 - 104
Nausea		Nausea present but not interfering with oral intake	Nausea leading to decreased oral intake	Nausea leading to minimal to no oral intake
Myalgia		Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Arthralgia		Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Headache		Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Fatigue		No interference with activity	Some interference with activity	Significant; prevents daily activity
Chills		Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Loss of appetite		Loss of appetite without decrease in oral intake	Decreased oral intake without weight loss	Decreased oral intake with weight loss
Malaise		No interference with activity	Some interference with activity	Significant; prevents daily activity

*This toxicity grading scale is adapted from CBER 2007b to enable ease of reporting by subjects in the diary cards for ‘patient reported’ solicited adverse events. This toxicity grading scale is a Sponsor standard that is used throughout the Influenza clinical programs for patient reporting. ‘Grade 4’ is not listed here but will be defined in the statistical analysis plan as necessary.

SPONSOR SIGNATURE PAGE

Protocol number: V89_18

Protocol title: A Phase 3 Randomized, Observer-Blind, Multi-center, Controlled Study to Evaluate Safety, Immunogenicity, and Lot-to-Lot Consistency of an Adjuvanted Cell Culture-Derived, H5N1 Subunit Influenza Virus Vaccine in Healthy Adult Subjects ≥ 18 years of Age

Document: Revised Protocol

Version: 3.0

Date: 15 MAR 2016

As an approver, I agree with the content and format of this document:

Name:

Role in trial:

Signature:

