An Exploration of a Prime-Boost Approach for Universal Influenza Vaccination: Immunogenicity and Safety Study of Inactivated Subunit H5N1 Influenza Vaccine in Prior Recipients of Live Attenuated H2N2, H6N1 and H9N2 Influenza Vaccines and in H5N1 and Live Attenuated Vaccine Naïve Individuals

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ABBREVIATIONS

Abbreviation	Definition
Ab	antibody
AE	adverse event
AI	avian influenza
BARDA	Biomedical Advanced Research and Development Authority
ca	cold-adapted (virus phenotype)
CBC	complete blood count
CFR	Code of Federal Regulations
CIR	Center for Immunization Research
CRIMSON	Clinical Research Information Management System of the NIAID
CSO	Clinical Safety Office
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
H1N1, etc.	hemagglutinin and neuraminidase subtypes of influenza virus
HA	hemagglutinin (influenza virus surface glycoprotein)
HAI	hemagglutination inhibition assay
HBsAg	Hepatitis B virus surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IDS	Investigational Drug Service
IgA	immunoglobulin A
IgG	immunoglobulin G
IM	intramuscular
IND	investigational new drug
IRB	Institutional Review Board
ISV	inactivated subvirion vaccine
LAIV	live attenuated influenza vaccine
LID	Laboratory of Infectious Diseases
MN	microneutralization assay
NA	neuraminidase (influenza virus surface glycoprotein)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PI	Principal Investigator
pISV	pandemic inactivated subvirion vaccine
pLAIV	pandemic live attenuated influenza vaccine
rRT-PCR	real-time reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SUSAR	suspected unexpected serious adverse reaction
$TCID_{50}$	50% tissue culture infectious dose
β-HCG test	test for human choriogonadotropin, an indicator of pregnancy

PROTOCOL SUMMARY

Protocol Title: An Exploration of a Prime-Boost Approach for Universal Influenza Vaccination: Immunogenicity and Safety Study of a H5N1 Influenza Vaccine in Prior Recipients of Live Attenuated H2N2, H6N1 and H9N2 Influenza Vaccines and in H5N1 and Live Attenuated Vaccine Naïve Individuals

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1.0	6 November 2018
1.1	19 November 2018
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2.0	27 February 2019

Subjects: Healthy male and non-pregnant female subjects 18-60 years of age

Number of Subjects: Approximately 32-35

Trial Design: Open-label outpatient trial of 1 dose of H5N1 pISV

Immunization Schedule:

Cohort	Number of Subjects	Previous Vaccine	Number of doses of inactivated H5N1 vaccine
1	12-15	2 doses of pandemic live attenuated influenza vaccine (pLAIV) H2N2, H6N1 or H9N2	1
2	20	Naive	1

Product Description: Monovalent Influenza H5N1 Subvirion Vaccine (A/VietNam/1203/04 (H5N1, clade 1) vaccine, lot number UD08916

1. INTRODUCTION

1.1 Background: Pandemic Influenza

Influenza is a negative-sense, single-stranded RNA virus belonging to the family *Orthomyxoviridae* that consists of 4 genera: influenza A, influenza B, influenza C, and Thogoto viruses. The proteins of influenza A are encoded by genes on 8 RNA segments. Influenza A viruses are widely distributed in nature and can infect a wide variety of birds and mammals, including humans. Influenza A virus subtypes are classified on the basis of the antigenicity of their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA).

Sixteen HA subtypes and 9 NA subtypes of influenza A viruses have been identified that infect birds. Waterfowl are the natural reservoir of avian influenza (AI) viruses. Most AI infections in birds are not associated with overt disease, and the viruses are generally thought to be in evolutionary stasis in these hosts. This is in contrast to influenza A viruses in the human population where relatively few subtypes have caused sustained outbreaks of disease: viruses bearing H1, H2, and H3 HA and N1 and N2 NA genes have circulated in the human population throughout the 20th century. H1N1 viruses first appeared in 1918 and circulated until 1957 when they were replaced by H2N2 viruses. These in turn were replaced in 1968 by H3N2 viruses that continue to circulate at the present time. In 1977, H1N1 viruses reappeared and have continued to co-circulate with the H3N2 viruses. The H1N1 virus circulating previously was replaced by the 2009 pandemic H1N1 virus. Influenza A and B viruses cause epidemics in humans each winter.

As seen in 2009, in addition to the seasonal influenza epidemics, the potential also exists for an influenza pandemic at any time. This occurs when an influenza strain with a novel HA subtype (with or without a novel NA subtype) appears in the human population. In the 20th century, pandemics occurred in 1918, 1957, and 1968, and were associated with significant morbidity and mortality (1). The 2009 H1N1 pandemic caused approximately 10,000 deaths in the United (2), 95% of whom were under the age of 65. It is estimated that, in the United States alone, the next influenza pandemic could cause 89,000 to 207,000 deaths, and 314,000 to 734,000 hospitalizations, as well as tens of millions of outpatient visits and illnesses, depending on the severity of the pandemic (3).

During the 2009 H1N1 pandemic, robust antibody responses to the HA stalk epitope were detected following infection or vaccination. This led Li and colleagues to propose that rare B cells directed against the conserved stalk epitope were amplified because the immunodominant head region of the HA was novel (4). Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory cells. Subsequently, Krammer and Palese have proposed that sequential immunization with influenza viruses bearing chimeric HAs with a conserved HA stalk and different HA head domains will amplify the antibody response to the HA stalk (5).

In three independent clinical trials, we have demonstrated that pandemic live attenuated influenza vaccine (pLAIV) establishes long lasting immune memory that is detected

when pLAIV recipients receive a dose of pandemic inactivated subvirion vaccine (pISV) (6-8).

However, we have not administered a pISV bearing group 1 HA to pLAIV recipients of a mismatched group 1 pLAIV. We have administered a group 1 pISV (H5N1 VN04) to recipients of an H7N3 pLAIV and a group 2 pISV (H7N7 Netherlands03) to recipients of an H2N3 pLAIV(6, 7). We have previously administered group 1 pLAIVs (H2N2, H6N1, H9N2) to study subjects in Baltimore (9-11). This study will examine the effect of a group 1 pISV (H5N1 VN04) vaccine to previous recipients of a different group 1 pLAIV recipients (H2N2, H6N1 and H9N2).

1.2 Vaccine Description

Monovalent Influenza Subvirion Vaccine (H5N1) is an inactivated unadjuvanted influenza vaccine. The vaccine is a sterile suspension prepared from influenza virus propagated in embryonated chicken eggs. The vaccine is manufactured by Sanofi Pasteur Inc., Swiftwater, PA. The virus-containing fluids are harvested and inactivated with formaldehyde. The influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a nonionic surfactant, polyethylene glycol p-isooctylphenyl ether (Triton® X-100), producing a "split virus". The split virus is then further purified by chemical means and suspended in sodium phosphate-buffered isotonic sodium chloride solution. The vaccine is formulated to contain 90 µg HA per 1.0 mL of the influenza virus strain A/Viet Nam/1203/04 (H5N1, clade 1). Porcine gelatin (500 µg per 1.0 mL) is added as a stabilizer. Thimerosal, a mercury derivative, is added as a preservative to a concentration of not more than 98.2 µg thimerosal per 1.0 mL (approximately 50 µg mercury per 1.0 mL). The vaccine may also contain residual amounts of formaldehyde (not more than 200 µg per 1.0 mL), polyethylene glycol p-isooctylphenyl ether (not more than 0.05%), and sucrose (not more than 2.0%). We will be administering 0.5 mL, or 45 µg HA per each subject.

The vaccine is provided as a suspension in 5 mL multi-dose vials and should be administered as an intramuscular (IM) injection. The vaccine should be stored between 2 to 8°C. The vaccine should not be frozen.

The vaccine is licensed for active immunization of persons 18-64 years of age at increased risk of exposure to the H5N1 influenza virus subtype contained in the vaccine. The clinical trial material for this study is a lot prepared specifically for investigational use, lot number UD08916. The vaccine will be provided by the Biomedical Advanced Research and Development Authority (BARDA), U.S. Department of Health and Human Services (DHHS). The product label is provided in Appendix D.

1.3 Clinical Experience with the H5N1 inactivated vaccine

A prospective, randomized, double-blinded, placebo-controlled, dose-ranging Phase 1-2 study was conducted in 451 healthy subjects aged 18-64 years (12). The vaccine was well tolerated. Mild pain at the injection site was the most common adverse event (AE) for all

doses of vaccine evaluated (90, 45, 15, or 7.5 μg). The number of subjects with an antibody response was highest among those who received two doses of 90 μg of vaccine: HI titers of ≥1:40 were observed in 58% of subjects in this group, and 54% of subjects in this group had microneutralization assay (MN) titers of ≥1:40. This vaccine was subsequently licensed for use in healthy adults aged 18-64 years of age at increased risk of H5N1 influenza infection (

Appendix C: H5N1 Vaccine Product Insert).

1.4 Rationale

Unadjuvanted inactivated H5N1 vaccines have been shown to be poorly immunogenic in humans, whether administered as inactivated or live attenuated vaccines (12, 13). Previously, it has been demonstrated that subjects who were primed with an inactivated H5N1 vaccine and then several years later receive another inactivated H5N1 vaccine developed significantly greater HI and MN antibody titers than unprimed subjects (14, 15).

In 2006 and 2007, the safety, infectivity and immunogenicity of 2 live attenuated H5N1 vaccines, H5N1 VN 04 cold-adapted (*ca*) and H5N1 HK 03 *ca*, were evaluated in healthy adults (13). Nineteen subjects received 2 doses of $10^{7.5}$ 50% tissue culture infectious dose ($TCID_{50}$) of H5N1 VN 04 *ca*. Of these subjects, 15 had vaccine virus detected by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) after either dose, however, only 10% of subjects had measurable antibody responses by HI assay, and 5% by MN assay. Fifty two percent of subjects had measurable anti-H5 serum immunoglobulin A (IgA) antibodies. Sixteen subjects received 2 doses of $10^{7.5}$ $TCID_{50}$ of H5N1 HK 03 *ca*. Vaccine virus was detected by rRT-PCR in 12 subjects after either dose, and none of the subjects had measurable HI or MN antibodies after vaccination. Three subjects had increases in serum anti-H5 serum IgA.

When the inactivated, unadjuvanted H5N1 subvirion vaccine was evaluated in healthy adults, 2 doses of 90 μg of vaccine were required to elicit a 4-fold or greater rise in either HI or MN titer against the A/Vietnam/1203/2004 (H5N1) virus in the majority of subjects (12). Other unadjuvanted H5N1 vaccines were also poorly immunogenic (reviewed in (16)). Inactivated and virosomal H5N1 vaccines, while poorly immunogenic, do elicit stalk antibody responses (17, 18).

Although initial immunization with the unadjuvanted inactivated H5N1 vaccine or with live attenuated H5N1 vaccines generally induced inadequate antibody responses, some data suggest that immune priming may occur which is only revealed when individuals are revaccinated with H5 vaccine. For example, Goji et al. demonstrated that subjects who had received a clade 0 inactivated H5N1 vaccine (derived from A/Hong Kong/156/97) were primed to respond years later to a clade 1 H5N1 vaccine (derived from A/VietNam/1203/04), and that their antibody responses to the clade 1 vaccine were substantially greater than those observed in H5-naïve subjects (14).

Previously we sought to determine whether the H5N1 VN 04 *ca* and H5N1 HK 03 *ca* vaccines induced subclinical priming that could be revealed when subjects who received these vaccines are immunized with the inactivated H5N1 subvirion vaccine. We found that use of the H5NI subvirion vaccine increased frequency of antibody response 82% by the hemagglutination inhibition assay and displayed a significantly higher antibody titer of 112 compared to 76 in naïve subjects. The affinity of antibody and breadth of cross-clade neutralization was also enhanced in the primed subjects.

We found (Table 1 below) that the head-specific antibody (Ab) response in pLAIV prime-pISV boost scenarios is not affected by the interval between pLAIV prime and pISV boost. We saw similar percent seroconversion and geometric mean titers (GMTs) in 3 independent clinical trials with different pLAIV/pISV pairs administered at different intervals, from 3 months to 56 months (6-8).

Table 1: Antibody Titers After pLAIV pISV Prime Boost Studies

pLAIV		pISV	Interval pLAIV-pISV	HAI Ab titer following receipt of pISV			
Vaccine	# doses			n	GMT (range)	Responders	
						%	GMT
H5N1 VN04	2	H5N1 VN04	56 m	11	48 (5-1280)	64	165
H7N7 NL03	2	H7N7 NL03	19-24 m	13	34 (2-1024)	69	119
H7N9 Anhui/13	2	H7N9 Anhui/13	3 m	14	74 (2-512)	79	175

HAI = hemagglutination inhibition assay

We have evidence that pLAIV primes for a stalk-specific response (19, 20). However, in each of our prime-boost studies, the inactivated vaccine boost that recalled the response primed by the pLAIV was matched for the HA head and as Halliley et. al. and Henry Dunand et. al. have shown, this approach boosts the head-specific antibodies to a much greater extent than the stalk-specific antibodies (19, 20). In the proposed study, our objective is to determine whether boosting with an inactivated vaccine bearing a mismatched head, but shared stalk will boost the stalk response preferentially. Preclinical studies have demonstrated that prime-boost strategies using influenza viruses with mismatched HA heads and conserved stalks boost the antibody response to the HA stalk (21, 22).

All participants in the study will have been exposed to group 1 stalk via exposure to H1N1pdm09 viruses since 2009. However, the subjects that received pLAIV 5-10 years ago differ from the control cohort in having received two doses of a different group 1 virus (H2N2 or H6N1 or H9N2) followed by interim exposure to H1N1pdm09 viruses and vaccines.

The first-time people were vaccinated against H1N1pdm09, they had a very robust stalk-specific response, presumably because the HA head was novel, and the stalk was conserved. However, on revaccination, the head-specific response became dominant and the stalk-specific response was not detected (23). These findings are consistent with Ellebedy et. al. findings that there was no rise in stalk-specific antibody titers over a 4-year period from 2010 to 2014 (18). The data from these studies indicate that (1) although stalk-specific immunoglobulin G (IgG) + memory B cells are detectable, they are minimally boosted by seasonal influenza vaccine that contains the H1N1pdm09 HA and (2) over time and re-exposure, the head-specific response dominates over the stalk-specific response.

The data from Ellebedy et. al. (18) suggest that it is likely that the H5N1 pISV will induce a stalk-specific response in the control cohort as well as the pLAIV primed subjects, but we hypothesize that recipients of the H2N2, H6N1 and H9N2 pLAIVs will have more memory B cells against the group 1 stalk than the control cohort because they differ from the control cohort in having received two doses of a different group 1 virus (H2N2 or H6N1 or H9N2). It is now 9 years since the emergence of the 2009 pandemic H1N1 virus and people would have been exposed to the virus or vaccine more than once so they will have a predominantly head-specific response to H1N1pdm09 as reported by Andrews et. al. (23).

Additionally, Yassine et. al. (24), reported that not all broadly neutralizing antibodies are created equal; using stalk stabilized probes against H1 seasonal, H1 pdm09, H2, H5 and H9 HA stems, they found that ~45% of 202 people had antibodies that bound the stalks of non-circulating viruses (H2, H5, H9) and 30% had antibodies that bound 4 of the 5 probes. They reported that sera collected either prior to the 2009 pandemic or afterwards showed no differences in cross-reactivity and they observed no difference in the profiles of cross-reactive sera from vaccinated and non-vaccinated subjects collected after the H1N1 pandemic emergence. However, there was a statistically significant boost in specific stalk antibody titers to H1 CA 09 HA SS probe in subjects that received pandemic H1N1 influenza vaccine compared to those that did not receive the vaccine within the same time period. They also noted a higher rate of cross-reactive antibodies in the older population, especially those born within or before the period of H2N2 pandemic (24).

We will use similar probes (25) to detect stalk antibodies in the sera from this trial and hypothesize that on administration of an H5N1 inactivated vaccine, we will see a stalk-specific antibody response in both cohorts but the magnitude of the response and the breadth of the stalk antibody response in the pLAIV primed subjects will be greater than in the controls.

In summary, we hypothesize that people that have been primed with pLAIVs bearing novel HAs will develop antibodies that react more broadly than people whose only exposure to a novel HA head was H1N1pdm09.

1.5 Participation of Children

It is felt that insufficient data are available to judge the potential risk in children. As such, children will not be enrolled in this study. In addition, to date, children have not received the H2, H6 or H9 *ca* vaccines.

1.6 Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation/Good Clinical Practice (ICH/GCP) guidelines, Food and Drug Administration (FDA) regulations (21 Code of Federal Regulations [CFR]), and the Johns Hopkins Bloomberg School of Public Health Institutional Review Board.

1.7 Hypothesis

Recipients of the H2N2, H6N1 and H9N2 pLAIVs will have more memory B cells against the group 1 stalk than the control cohort, whose only exposure to a novel HA head was H1N1pdm09. Therefore, the magnitude of the response and the breadth of the stalk antibody response in the pLAIV primed subjects will be greater than in the controls following administration of an H5N1 inactivated vaccine.

2. OBJECTIVES

2.1 Primary Objective:

To explore the difference in the stalk antibody response against Influenza A group 1 stalks as measured by enzyme-linked immunosorbent assay (ELISA) and neutralization titer after receipt of the Monovalent Influenza Subvirion Vaccine (H5N1) in previous recipients of H2N2, H6N1 and H9N2 pLAIVs compared with healthy, pLAIV-naïve individuals.

2.2 Secondary Objective:

Assess the reactogenicity of the Monovalent Influenza Subvirion Vaccine (H5N1) in previous recipients of live attenuated influenza vaccine (LAIV) compared with healthy, pLAIV-naïve individuals.
Adverse events will be assessed, graded and recorded for 28 days after vaccination. Serious Adverse Events (SAEs) will be assessed for 90 days after the immunization.

2.3 Exploratory Objectives:

- To assess the biological activity of antibodies to the HA stalk region by passive transfer of serum from study subjects to mice to assess their ability to protect the mice from lethal challenge with an influenza virus.
- To correlate ELISA and neutralizing stalk antibody titers with functional ability of the post vaccine serum to protect mice from lethal challenge.
- To assess the breadth of the vaccine induced antibody response.
- To assess plasmablast and memory B cell responses to the pISV in the previous pLAIV recipients compared with naïve individuals.
- To assess the longevity of the stalk antibody response.
- To determine the titer of anti-H5 head antibodies in the 2 cohorts of subjects.
- To determine the titer of antibodies directed to the HA head received in the pLAIV doses.

3. STUDY DESIGN

3.1 Summary

This open-label, outpatient, exploratory study will recall study subjects who previously received a group 1 pLAIV (H2N2, H6N1 or H9N2) and administer 1 dose of pISV (e.g. H5N1 pISV) to determine whether the HA stalk antibody response is amplified following administration of pISV H5N1 with a mismatched HA head and conserved HA stalk.

A control cohort of naive subjects will receive H5N1 pISV.

All subjects will be followed for approximately 360 days after receipt of the H5N1 pISV.

3.2 Study Schedule

The study will be divided into 3 phases:

1. Screening, eligibility determination and consent up to 60 days prior to vaccination/enrollment (usually 2 visits initiated under a separate institutional review board (IRB)-approved screening protocol)
2. Vaccination/Enrollment (Day 0)
3. Outpatient follow-up at 7, 14, 28, 56, 90, and 360 days post vaccination

3.3 Study Procedures

(See Section 6 for detailed description of study procedures)

3.4 Sample Size

Up to 35 healthy adults will be selected to receive 1 dose of H5N1 pISV. Up to 15 of these subjects will have previously received group 1 pLAIVs (H2N2, H6N1 or H9N2), and 20 of these subjects will be naïve. More than 35 subjects may be consented, vaccinated and enrolled (see Replacement of Subjects) if necessary to achieve enrollment goals. The Medical Monitor/Safety Office and the Clinical Trials Monitor will be notified promptly by email in advance if the study team anticipates that study vaccinations will exceed 38 subjects per replacement procedure.

3.5 Treatment assignment

All subjects enrolled in the study will receive 1 dose of H5N1pISV

3.6 Duration of Participation

Duration of individual subject participation in the trial is approximately 360 days from time of vaccination (not including 1 – 2 screening visits prior to enrollment) and will consist of a vaccination/enrollment visit and 6 follow up visits. The duration of the study, from the time of the first subject vaccination to the end of the last subject study visit, is estimated to be approximately 18 months.

4. SELECTION OF STUDY SUBJECTS

Individuals who previously received H2N2, H6N1, or H9N2 pLAIV as participants in CIR vaccine studies and who indicated they would be willing to participate in future studies, will be contacted and invited to participate in this study, as described in Section 6. Healthy subjects will be recruited from the general population for the LAIV negative controls.

4.1 Inclusion Criteria

All the following criteria must be met for a subject to be enrolled in this trial:

1. Adult males and non-pregnant females between 18 years and 60 years of age inclusive.
2. General good health, without significant medical illness, physical examination findings, or significant laboratory abnormalities as determined by the investigator.
3. Available for the duration of the trial.

4. Able to demonstrate understanding of key study concepts, study rationale, and study participation requirements by scoring ≥ 70% on a written comprehension assessment in ≤ 3 attempts.
5. Willingness to participate in the study as evidenced by signing the informed consent document.
6. Willingness to allow storage and testing of laboratory samples for future research.
7. Received 2 doses of live attenuated H2N2, H6N1, or H9N2 vaccine in a prior trial (Cohort 1) or H2N2, H6N1 and H9N2 naïve (Cohort 2)
8. Willingness to forego seasonal influenza virus vaccination from 1 month before vaccination until 3 months after vaccination.
9. Female subjects of childbearing potential must agree to have used effective birth control methods beginning at least one month prior to vaccination, and continuing with 'per label/fully effective use' for the chosen method for duration of the study, from amongst these:
    - pharmacologic/hormonal contraceptives, including oral, parenteral, subcutaneous, and transcutaneous delivery;
    - condoms with spermicide;
    - diaphragm with spermicide;
    - intrauterine device;
    - absolute abstinence from heterosexual intercourse as a matter of normal preferred lifestyle;

    or must be surgically sterile or must be age 50 AND have had no menses at all for at least one full year.

    All females must provide urine for pregnancy testing prior to enrollment (immediately prior vaccination), as well as a statement of menstrual history and a summary of all potentially reproductive sexual activity for the month prior to vaccination, and at each study contact throughout the study, and report known or suspected pregnancy immediately.
10. Willingness to refrain from blood donation during the course of the study.

4.2 Exclusion Criteria

The presence of any one of the following criteria is sufficient to exclude a prospective subject from enrolling in this study:

1. Pregnancy as determined by a positive test for human choriogonadotropin (β-HCG), an indicator of pregnancy or history of recent unprotected intercourse in a woman of reproductive capacity.
2. Currently breast-feeding.
3. Evidence of clinically significant neurologic, cardiac, pulmonary, hematologic, hepatic, rheumatologic, autoimmune, or renal disease by history, physical examination, and/or laboratory studies.
4. Behavioral or cognitive impairment or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and cooperate with the study protocol.

5. Have medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
6. Other condition that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial or would render the subject unable to comply with the protocol.
7. History of anaphylaxis in response to any vaccine or vaccine component.
8. History of life-threatening reaction to prior influenza vaccine.
9. Positive enzyme-linked immunosorbent assay (ELISA) and confirmatory test for human immunodeficiency virus type 1 (HIV-1).
10. Positive ELISA and confirmatory test (e.g., HCV RNA PCR) for hepatitis C virus (HCV).
11. Positive hepatitis B virus surface antigen (HBsAg) by ELISA.
12. Known immunodeficiency syndrome or history suggestive of impaired immune function.
13. History of Guillain-Barré syndrome.
14. Use of chronic oral or intravenous administration (≥14 days) of immunosuppressive doses of steroids, i.e., prednisone >10 mg per day, immunosuppressants or other immune-modifying drugs within 30 days of starting this study.
15. Receipt of a live vaccine within 4 weeks or a killed vaccine within 2 weeks prior to study vaccination.
16. Receipt of blood or blood-derived products (including immunoglobulin) within 6 months prior to study vaccination.
17. Receipt of another investigational vaccine or drug within 30 days prior to study vaccination.

In addition to the above, participants in Cohort 1 (pLAIV recipients) and Cohort 2 (naïve cohort) must also not experience any of the following:

1. Previous enrollment in an H5N1 influenza vaccine trial.

Participants in Cohort 2 (naïve cohort) will be excluded if they are:

1. Seropositive to the H5N1 influenza A virus (serum hemagglutination inhibition assay (HAI) titer >1:8).
2. Have previously received an investigational LAIV in another study.

4.3 Access to Medical Records

A medical history will be obtained directly from each subject. Medical records will not be requested unless there is a need to clarify a question in the subject's medical history or to document an AE during the study. Medical records will not be requested without a signed medical release and the informed consent of the subject.

4.4 Enrollment

Subjects will be scheduled for vaccination when they complete the screening process, are determined to be eligible, and provide consent for study participation. Subjects will be considered enrolled in the study upon receipt of the study agent.

4.5 Subject Withdrawal/Termination Criteria

A subject will have completed the study if he/she receives the specified study vaccination and completes at least the Day 90 visit after the vaccination. A subject will be withdrawn from the study and not be considered to have completed the trial if any of the following apply:

1. Research terminated by Sponsor or investigator: applies to the situation where the entire study is terminated by the Sponsor or investigator for any reason.

2. Withdrawal of consent: applies to a subject who withdraws consent to participate in the study for any reason.

3. Noncompliant with protocol: applies to a subject who does not comply with protocol specific visits, evaluations, or procedures on a consistent basis, such that adequate follow-up is not possible, the subject's safety would be compromised by continuing in the trial, or the integrity of the study data could potentially be harmed.

4. Withdrawal by PI: may occur if the investigator believes that it is in the best interest of the subject to withdraw the subject.

5. Other: a category used when previous categories do not apply and requires an explanation.

4.6 Replacement of Subjects

Subjects who are enrolled, vaccinated and subsequently withdraw, are withdrawn, or are taken off study prior to completion of Day 28 follow up visit, may be replaced subject to the principle investigator's discretion in order to ensure sufficient data to achieve study objectives. All vaccinated subjects will be followed for safety if subject circumstances allow. All vaccinated subjects will be followed for safety if subject circumstances allow. See 'Sample Size', Section 3.4, for sponsor/safety notification requirement related to replacement.

4.7 Special Situations

1. *Pregnancy:* If a subject becomes pregnant, she will be advised to notify her obstetrician of study agent exposure. The subject will not be included in the immunogenicity evaluations from her estimated date of conception even if the pregnancy ends early. If the pregnancy occurs prior to Study Day 90, she will be encouraged to remain in the study for periodic safety evaluations and will be followed until completion of her pregnancy or the end of the study. The subject will be asked to sign a release of medical information form so that records can be obtained from her pregnancy provider regarding the outcome of the pregnancy. If a subject becomes pregnant after Study Day 90, but before Study Day 360, she will be withdrawn from the study. (See Investigator Reporting Responsibilities – Section 8.5).

2. *Lost to follow-up:* A subject who cannot be contacted by telephone or other means of communication, including U.S. mail, is considered lost to follow-up. A subject may be

considered lost to follow-up and withdrawn from the study once 3 attempts to contact the subject by any means and a certified letter sent to the last known address have failed to elicit a response from the subject.

3. *Incarceration***:** If a subject becomes incarcerated during the course of the study, he or she may be terminated from the study if the period of incarceration renders him or her unable to complete the study. Alternatively, a subject who received the investigational product and was subsequently incarcerated may be taken "off treatment" rather than withdrawn to facilitate continued safety monitoring upon release.

5. VACCINE PREPARATION

5.1 Supplies

Monovalent Influenza Subvirion Vaccine (H5N1) vaccine, lot number UD08916, will be supplied in multiuse vials by BARDA. Vaccine for this protocol will be stored at the Division of Microbiology and Infectious Diseases (DMID), National Institutes of Health (NIH), repository until requested by the Center for Immunization Research (CIR). Vaccine will be formally requested to be transferred to the Pharmacy and Investigational Drug Service (IDS), Room 100 Osler at the Johns Hopkins Hospital by the Principal Investigator (PI) after IRB and FDA approval for the study has been granted. Vaccine will then be stored at the IDS. On the day of vaccination, the PI or disignated clinical investigator will supply to the pharmacy individual participant specific vaccine request form (prescription) for each subject to be vaccinated. Each individual subject prescription will include the protocol number, the vaccine name, the vaccine dose, the investigational new drug (IND) number, and the number and name of the subject to be vaccinated. The IDS requires that the name be included and keeps these requests in a locked file as part of the study documents. The Pharmacist will draw up the vaccine from the multi-dose vials into syringes, 0.5 mL vaccine/syringe (45 μg dose, 10 per vial) based on the number of subjects to be vaccinated. The pharmacist will label each syringe with the study product name, PI initials, study number, subject number, preparer and study product verifier's initials and expiration date and time. He or she will supply the appropriate number of syringes to the investigator. The vaccine will be transported to the investigator on wet ice or using cool packs for administration at the vaccination site on the same day.

5.2 Vaccine Storage and Accountability

Vaccine will be stored at 2° to 8°C. **Vaccine should not be frozen.** Vaccine will be shipped to the IDS from the DMID repository with cool packs and a temperature log to ensure that the vaccine remained within the acceptable range. Vaccine cannot be used until it is verified by the Sponsor that the temperature has stayed within range. If during shipping, the vaccine falls out of the acceptable range, it will be quarantined until BARDA has been contacted and has determined whether or not it is acceptable to use the shipment. If not, it will be disposed of per BARDA's instruction, and a new shipment will be requested. Once at the IDS, vaccine will be stored in temperature-monitored refrigerator, and the logs will be available for the monitors, if they wish to see them. In transport from the IDS, a study specific procedure re: "The transport of Investigational Agent for administration to study subjects" will be followed.

5.3 Disposition of Used/Unused Supplies

At the time of study product administration, the study product administrator and verifier will initial the label and remove one of two detachable labels from the syringe and place it on a piece of paper (labeled with the CIR study number, the subject's study ID number, and the time of virus administration). The paper with the labels will be kept in the Study Binder. The other label will be placed in the subject chart as source documentation. The study product administration time will be documented on the Study Product Administration Record (SPAR). A copy of the completed SPAR will be placed in the Study Binder. The original will be returned to the CIR laboratory personnel along with any unused supply. The SPAR will be maintained in the laboratory and filed in the accountability binder. Used syringes will be disposed of in a sharps container. Any unused syringes will be returned to the pharmacy with the labels intact and will be documented on the accountability log by the pharmacist as unused. The Pharmacist will also document the use of multi-dose vials. All open multi-dose vials and unused syringes will be destroyed by the pharmacy according to pharmacy specific procedures. Unopened vials will be kept by the pharmacy until instructed by the Sponsor. In this manner, monitoring personnel will be able to account for all vials and syringes used for the study.

6. STUDY PROCEDURES

6.1 Recruitment and Screening (Up to 60 Days Prior to Vaccination)

Subjects will be evaluated, initially, through a JHBSPH IRB-approved general screening protocol – JH200 (IRB# H22040219A2). This general screening protocol is used to identify subjects who may be eligible to participate in a vaccine trial.

6.2 Recruitment

The CIR disseminates information about opportunities to participate in its vaccine clinical trials via IRB-approved content on its own website (centerforimmunizationresearch.org), social media platforms, YouTube, and in web, newspaper, radio and television advertisements. Study specific IRB-approved fliers and brochures are distributed locally.

Cohort 1 will consist of prior recipients of Live Attenuated H2N2, H6N1 and H9N2 Influenza Vaccines as participants in previous CIR studies (CIR 211, CIR 247, CIR 251) With each initial and subsequent telephone contact, subjects are asked if they will allow us to call them about opportunities to participate in future studies. The response to this question is documented on a pre-screening assessment form and in the CIR Adult Studies Data Base. Study staff has compiled a list of approximately 63 volunteers who participated in these three studies from 2005 - 2009. They will be contacted via phone, and/or IRB-approved letter, and invited to participate in our general screening study to see if they would be eligible and willing to participate in this study as recalled subjects. After this initial contact, subjects will be referred to the telephone recruiter. The recruiter will administer a brief qualifying phone screen after obtaining verbal consent and eligible recruits will be scheduled for in-person screening visits according to their interests and availability.

Cohort 2 – the control cohort consisting of LAIV-naïve/H5 antibody negative subjects, will be identified as individuals who make inquiries by telephone call or email to the clinical trial site. A telephone recruiter will respond to calls and emails and provide brief scripted descriptions of currently accruing CIR trials as specified in the CIR's general screening protocol. The recruiter will administer a brief qualifying phone screen after obtaining verbal consent and eligible recruits will be scheduled for in-person screening visits according to their interests and availability.

The screening evaluation outlined below will be completed within 60 days prior to vaccination. During the initial in-person screening visit trained study staff will:

1. Provide a copy of the screening study consent form and encourage the volunteer to read the consent and ask questions
2. Administer a brief, written comprehension assessment to confirm understanding of the screening study procedures and requirements
3. Obtain written, informed consent from the subject indicating willingness to participate in the CIR JH200 Screening Protocol as evidenced by subject signature, date and time on the informed consent document. The staff person obtaining consent will sign, date and time the ICF document. A copy of the signed document will be given to the subject. The original will be kept in the subject chart.

4. Elicit a complete medical history, including current medications, and, for females, menstrual and contraceptive history, assessment of childbearing potential, and/or history of surgical sterility or menopausal status

5. Provide human immunodeficiency virus (HIV) pretest counseling, including information about HIV testing, transmission, and prevention, explanation of test results, post-test counseling, results reporting, and that testing is voluntary, but necessary for study participation

6. Provide pregnancy prevention counseling for females focused on the importance of preventing pregnancy during study participation, potential risks associated with pregnancy while taking investigational drugs, effective contraceptive options, and local resources for obtaining family planning services.

7. After review of medical history, the study nurse or nurse practitioner will offer at no cost the regular dose quadrivalent seasonal flu vaccine – Fluzone.

Additionally, the following activities may occur or be deferred to a subsequent screening visit depending on study requirements:

1. Administer a complete physical examination

2. Obtain blood for complete blood count (CBC) with differential and platelet count, hepatitis B surface antigen, hepatitis C antibody, HIV antibody (will be completed within 30 days of vaccination) and H5N1 HAI assay. Urine will be collected from females for ß-HCG testing.

6.3 CIR 327 Study-Specific Screening and Informed Consent

After the initial screening process has been completed, Subjects will be notified of their eligibility for further screening. All clinically significant abnormalities will be reviewed with the subjects and referrals for follow-up care will be provided. Subjects who test positive for hepatitis B, hepatitis C, or HIV) will be notified and referred to the Baltimore City Health Department according to Maryland reporting requirements. Eligible and willing subjects will be asked to return to the CIR for study-specific screening and consent.

Informed consent is an ongoing process that includes the informed consent document and continues throughout the trial. Trained study staff will initiate the consenting process as follows

1. Potential study participants will be given a copy of the IRB-approved study consent document to read.

2. Subjects will be encouraged take as much time as they need and to ask questions

3. Subjects will be asked to complete multiple-choice/fill in the blank questionnaire to evaluate comprehension of study aims, procedures, requirements, and risks.

4. Subjects will meet privately with study staff to review the completed comprehension assessments to identify gaps in subjects' understanding of study rationale, requirements, and risks, and their role as study participants.

5. Study staff will review the pertinent study information with the subject until the subject verbalizes understanding. The subject may be offered up to 2 more attempts to answer 70% or more of the questions correctly.

6. Subjects achieving a score of 70% or better and verbalizing willingness to participate in the study will be asked to indicate their consent in writing by signing the informed consent document and recording date and time that consent was given.

7. **The staff person obtaining consent will sign, date and time the ICF document. A copy of the signed document will be given to the subject. The original will be kept in the subject chart.**

After obtaining informed consent the following procedures may be completed

1. A complete physical exam if not done during the general screening visit or at the discretion of the PI or designee.

2. No cost flu vaccine will be offered to subjects who declined flu vaccination during initial screening. Subjects who continue to decline the flu vaccine will be counseled as to the benefits of annual seasonal flu vaccine and risk of illness due to declining or delaying vaccination. Subjects will be offered the option of deferring study enrollment for 30 days if they choose to accept the flu vaccine.

3. Any laboratory assays or screening procedures mentioned above that were not completed during the first screening visit (or if assays need to be repeated), may be completed during the study specific screening visit. Urine will be collected from females for ß-HCG testing.

6.4 Immunization Procedure

Syringes prepared by the IDS and placed in cold box (as per Section 5.1 above) will be removed in order as subjects receive vaccination. The syringe will be warmed briefly, the subject number will be verified, and then the vaccine will be administered intramuscularly (IM) in the deltoid muscle to eligible and consenting subjects. Empty syringes will be disposed of in an appropriate sharps container.

6.5 Clinical Evaluation

Vital signs (temperature, heart rate, respiratory rate, and blood pressure) will be assessed during screening, pre and 30 minutes post vaccination, Day 7 and Day 14. Focused physical examinations (examination of head, eyes, ears, nose, throat, heart and lungs) will be performed on the day of vaccination and at Day 7 and as indicated by interim history to day 360.

6.6 Treatments that Could Potentially Interfere with Vaccine-Induced Immunity

The following criteria will be reviewed with the subjects during each follow up visit up to and including day 90. If any of these become applicable during the study, it will be noted in the subject's record. The subject will not be included in further immunogenicity evaluations as of the visit in which the exclusionary treatment was reported. Subjects will be encouraged to participate in the safety evaluations, including blood draws at the discretion of the investigator for the duration of the study.

1. Use of any investigational drug or investigational vaccine other than the study article within 90 days of receipt of the study agent.

2. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs (topical and nasal steroids are allowed).

3. Receipt of any licensed vaccine in the 4 weeks after vaccination.

4. Receipt of immunoglobulins and/or any blood products.

6.7 Concomitant Medications

A concomitant medication (con-med) is a drug or biological product, other than a study drug, taken by a subject during a clinical trial. Information about routine medication use will be documented in the screening medical history. Concomitant medications and changes in pre-existing medication regimens indicating new diagnoses or worsening of a pre-existing condition will be collected from vaccination to Day 28 to identify solicited and unsolicited AE's, new or changed significant or chronic conditions and treatments potentially interfering with vaccine immunity. Between day 28 and 90 concomitant medications will only be collected if associated with an SAE or with a treatment that interferes with vaccine-induced immunity as above.

6.8 Study Procedures By Day

See Appendix A for a tabular representation of study procedures. In the description below, Study Day 0 is the day of receipt of vaccine. The days are numbered thereafter.

Study Day 0 (Day of Vaccination/Enrollment)

1. Verify that study-specific Informed Consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Record vital signs (blood pressure, temperature, heart rate, and respiratory rate).
4. Perform an abbreviated history and focused physical examination.
5. For women, obtain urine for β-HCG testing. Ensure the test is negative before proceeding; a positive test will exclude the subject from the trial.
6. Obtain blood for Serum and for PBMCs.
7. Administer the vaccine.
8. Observe for at least 30 minutes after vaccination to evaluate for immediate adverse reactions.
9. Record vital signs (blood pressure, temperature, heart rate, and respiratory rate) 30 minutes after vaccination.
10. Review pregnancy prevention Counseling (Females only).

Study Day 7 ±1 Day

1. Obtain interim history and perform focused physical examination, concentrating on any acute complaints and adverse events after vaccination.
2. Assess continuing eligibility and for treatments that could potentially interfere with vaccine-induced immunity**.**
3. Record vital signs. (blood pressure, temperature, heart rate).
4. Evaluate injection site.
5. Perform an abbreviated history and focused physical examination.
6. Obtain blood for serum and for PBMCs.
7. Review Pregnancy Prevention counseling (Females only).

Study Day 14 ±2 Day

1. Obtain interim history concentrating on any acute complaints.

2. Assess continuing eligibility. Review use of any concurrent medications. Assess for treatments that could potentially interfere with vaccine-induced immunity.
3. Perform focused physical exam if indicated.
4. Record vital signs. (blood pressure, temperature, heart rate).
5. Obtain blood for serum and PBMCs.
6. Review pregnancy prevention counseling (Females only).

Study Day 28 ±7 Days

1. Obtain interim history concentrating on any acute complaints.
2. Assess continuing eligibility. Review use of any concurrent medications. Assess for treatments that could potentially interfere with vaccine-induced immunity.
3. Perform focused physical exam if indicated.
4. If female, obtain urine for β-HCG testing.
5. Obtain blood for serum and PBMCs.

Study Day 56 ±7 Days

1. Perform basic history, concentrating on any acute complaints.
2. Obtain blood for serum and PBMC's.

Study Day 90 ±14 Days

1. Perform basic history, concentrating on any acute complaints.
2. Obtain blood for serum and PBMCs.
3. If female, obtain urine for β-HCG testing

Study Day 360 ±30 Days

1. Perform basic history, concentrating on any acute complaints.
2. Obtain blood for serum and PBMCs.

6.9 Clinical and Research Laboratory Testing

For both cohorts, the maximum volume of blood to be drawn is approximately 755 mL over the 12 month study period and not more than 550 ml's in any 8 week period, which should not compromise the health of trial subjects and is consistent with the NIH guidelines set forth in the M95-9 (rev) "Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center ."

Using standard techniques, and with samples labeled with a unique study identifier for each subject, Quest Laboratories will perform the following tests:

1. Complete blood count plus white blood cell differential and platelets.
2. Chemistry panel (if indicated).
3. HIV assay (4th generation ELISA with Western blot confirmation).
4. HBsAg ELISA

5. HCV RNA PCR confirmation.
6. Serum pregnancy test (if indicated)

Urine β-HCG testing will be performed at the clinical trial site using an FDA-approved urine pregnancy test kit.

HAI assays done for the purpose of screening potential subjects will be done by the CIR laboratory.

Quantitative ELISA and MN for anti-stalk antibody, the passive serum transfer study, and the LD50 challenge study in mice will be assessed at the Icahn School of Medicine at Mount Sinai.

Assessment of Plasmablasts and Memory B cells responses will be conducted at the Vaccine Research Center, NIH.

De-identified PBMCs may be sent to Adaptive Biotechnology for immune profiling.

Nasal secretions will be obtained by nasal lavage using 20 mL of Lactated Ringer's Solution for Injection (10 mL per nares) with the glottis in a closed position. The collected lavage secretions will be used to screen for adventitious agents should subjects report URI symptoms during the 28 day post-vaccination AE reporting period.

Specimens will be stored at the clinical trial site or the Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Diseases (NIAID). Access to research samples will be limited using a locked room. Samples and data will be stored using codes assigned by the investigators. Laboratory data will be kept in password-protected computers. Only investigators will have access to the samples and data. Samples at the clinical trial site are tracked manually on specimen logs that are kept in a separate, locked file cabinet.

Any loss or unanticipated destruction of samples that compromises the scientific integrity of the data collected for the study (for example, due to freezer malfunction) will be reported to the IRB.

6.10 Testing of previous samples

Samples from the subjects in the previous pLAIV studies are still stored in the NIAID repository. With subject consent, pre-and post pLAIV serum samples will be retrieved and tested for anti-stalk antibodies, in parallel with the samples collected during this study.

7. RETENTION OF SPECIMENS FOR FUTURE USE

All specimens collected as part of this trial may be stored indefinitely for future research. These samples may be used to learn more about influenza infection and other diseases but will not be used for genetic testing. These samples will not be sold or used to make commercial products. Samples will be stored only with the subject's permission, which is

a requirement for participation in the study. The subject may withdraw permission for future use of specimens at any time. If a subject withdraws his or her permission for future use of specimens, those specimens will be destroyed. All samples stored will be labeled with the subject's study identification (ID) number, which cannot identify the study subject but is linkable to other research databases (e.g., from questionnaires, clinical assessments, logbooks, etc.) generated by the main study. The database will contain only the study subject's ID number. A master log linking the study subject ID number to the name of the subject will be maintained in a password protected database system with access limited to authorized research team members**.**

At the completion of the protocol (termination), samples and data will either be destroyed, or transferred to a repository (JHBSPH IRB protocol R22.05.04.29.A2). In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval. The research use of stored, unlinked, or unidentified samples (for example, as a standard for immunological analyses) may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research, which is authorized to determine whether a research activity is exempt.

8. SAFETY AND ADVERSE EVENT REPORTING

8.1 Definitions

8.1.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in an investigational subject following receipt of an investigational drug, regardless of causality. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), physical exam, symptom, or disease temporally associated with the use of the investigational vaccine, whether or not related to it. Exacerbation of pre-existing conditions and intercurrent illnesses will be recorded as AEs. Stable chronic conditions that are present prior to enrollment and do not worsen are not considered AEs but will be accounted for in the subject's medical history. If a diagnosis is clinically evident or subsequently determined, the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

A laboratory abnormality that is not included in the solicited AEs will be reported as an AE if it results in an intervention. Interventions include, but are not limited to, treatment discontinuation, dose reduction/delay, additional assessments (including repeat testing at a later time point if not specified in this protocol), or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an AE, at the discretion of the investigator. This could include a laboratory result for which there is not an intervention, but the abnormal value suggests a disease or organ toxicity. If the laboratory abnormality is the clinical sequela of a clinical condition, the diagnosis or

medical condition should be reported as the AE (e.g., renal failure, hematuria) rather than the laboratory abnormality (e.g., elevated creatinine, urine red blood cell increase).

8.1.2 Solicited Adverse Events (Protocol-Specified Adverse Events)

AEs will be categorized as Solicited AEs and Other AEs. Solicited AEs are predefined AEs that can potentially occur after vaccine administration. Reactogenicity events are solicited adverse events that occur in the first 7 days after vaccination. The following will be considered solicited AEs for subjects receiving the Monovalent Influenza Subvirion Vaccine (H5N1):

1. Fever ≥100.4°F (38°C) oral
2. Headache
3. Malaise
4. Nausea
5. Myalgia
6. Injection pain
7. Injection site erythema
8. Injection site swelling
9. Injection site induration
10. Injection site pruritus

8.1.3 Unsolicited Events

AEs that are not specifically listed in Section 8.1.2 and not documented in the screening medical history as pre-existing conditions will be categorized as "Other AE's and identified by asking subjects about any new diagnoses, acute illnesses, worsening of a pre-existing condition, visits to the physician, ER, or hospital, medication use and indication.

8.1.4 Adverse Reaction (AR)

An AE that is caused by the vaccine.

8.1.5 Suspected Adverse Reaction (SAR)

An AE for which there is a reasonable possibility that the vaccine caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the vaccine and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which implies a high degree of certainty.

8.1.6 Serious Adverse Events

A SAE is an AE, whether considered related to the investigational vaccine or not, resulting in one of the following outcomes:

1. Death during the period of protocol-defined surveillance.
2. Life threatening event: defined as an event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe.

3. Requires hospitalization or prolongation of a hospitalization during the period of protocol-defined surveillance: defined as at least an overnight stay in the hospital for treatment that would have been inappropriate if administered in the outpatient setting.
4. Results in a congenital anomaly or birth defect.
5. Results in a persistent or significant disability or substantial disruption of the ability to conduct normal life functions.
6. Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious AE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.7 Unexpected Adverse Events

An AE is considered unexpected if it is not listed in the Investigator Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. "Expected" means that the event has previously been observed with the investigational agent and is identified and/or described in the current Investigator's Brochure (IB) or package insert. It does not mean that the event is expected with pharmacologically similar drugs, the underlying disease(s), or concomitant medications. Such events are considered unexpected for reporting purposes.

8.1.8 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

8.1.9 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

8.1.10 Serious Unanticipated Problem

A UP that meets the definition of a serious adverse event or compromises the safety, welfare or rights of subjects or others

8.1.11 Unanticipated Problem that is Not an Adverse Event (UPnonAE)

A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

8.1.12 Serious Protocol Deviation

A "Protocol Deviation" [see below] that meets the definition of a Serious Adverse Event or compromises the safety, welfare, or rights of subjects or others.

8.1.13 Protocol Deviation

Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur but cannot be prevented.
3. Those that are discovered after they occur.

8.1.14 Non-compliance [Definition is IRB dependent]:

At a Site Overseen by non-NIH IRB: Investigators must follow the definitions, requirements, and reporting procedures per the IRB of record for the study/site where the possible non-compliance event occurred.

8.2 Recording Period for Adverse Events

- All non-serious AEs will be assessed, recorded and reported for 28 days beginning at study vaccination, and will be followed to resolution, even if more than 28 days, or until the PI deems the event to be chronic or the subject to be stable.
    - Solicited non-serious AEs may be assessed/graded per a different table, if specified herein, and may be recorded and reported separately for tracking purposes.
    - Reactogenicity AEs/events are solicited AEs that are collected in the first 7 days after vaccination and may be recorded and reported separately for tracking purposes.
- SAEs, regardless of causality, will be assessed, recorded and reported for 90 days beginning at study vaccination, and will be followed to resolution, even if more than 90 days, or until the PI deems the event to be chronic or the subject to be stable.
- SUSARs, UPs, and UPnonAEs will be assessed, recorded and reported through the end of the study, beginning at study ENROLLMENT, and will be followed to resolution, even if beyond the end of the study (as may be permissible and possible in consultation with the IRB and sponsor) or until the PI deems the event to be chronic or the subject to be stable.

Assessment of safety will include clinical observation and monitoring of clinical and immunologic parameters. Safety will be evaluated by monitoring of subjects for local and systemic adverse reactions within the first 30 minutes post vaccination. Subjects will return to the clinic on Day 7 following vaccination for in-person clinical assessment.

All AEs will be graded for severity and assessed for relationship to the study product. A study clinician will be available 24 hours a day during the study evaluation period. Should a subject call a study clinician to report an AE, it will be fully documented in the subject's study chart and discussed with the PI. Interim follow-up visits (between protocol scheduled visits) may be scheduled at the PI's discretion if additional assessment is indicated.

All AEs will be captured in the source documents and in Clinical Research Information Management System of the NIAID (CRIMSON). Those assessed as serious will be further reported on the Sponsor's SAE-UP report form. The AEs judged to be possibly, probably, or definitely related to the study product will be followed as specified above, generally until resolution.

8.3 Association of Adverse Events with Receipt of Study Vaccine

All AEs will have their possible relationship to study vaccine. Causality will be assessed by the PI or designee using the following definition:

Definitely related:	Clear-cut temporal association, and no other possible cause.
Probably related:	Reasonable temporal association and a potential alternative cause is not apparent.
Possibly related:	Less clear temporal association; other causes also possible.
Unlikely related:	Temporal association between the AE and the vaccine or the nature of the event is such that the vaccine is not likely to have had any reasonable association with the observed illness/event (cause and effect relationship improbable but not impossible).
Not Related:	The AE is completely independent of vaccine administration; and/or evidence exists that the event is definitely related to another cause.

The degree of certainty with which an AE can be attributed to administration of the study vaccine will be determined by how well the event can be understood in terms of 1 or more of the following:

1. The event being temporally related with vaccination.
2. A reaction of similar nature having previously been observed with this type of vaccine and/or formulation.
3. The event having often been reported in the literature for similar types of vaccines.

8.4 Assessment of Severity

All AEs will be graded for severity.

- **Solicited AEs will be assessed using the grading system shown in Appendix B: Local and Systemic Adverse Event Grading Systems. This table is based on the NIAID "Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events," Version 2.1, March 2017: https://rsc.tech-res.com/docs/default-source/safety/daids-ae-grading-table-**

mar2017.pdf.

- **All AEs will be assessed by the study investigators using the grading system shown in Table 2, below:**

Table 2: Adverse Event Grading System

Severity	Grade	Defined
None	0	N/A
Mild	1	No effect on activities of daily living, over the counter treatment given ≤2x/day
Moderate	2	Partial limitation in activities of daily living (can complete ≥50% baseline, or treatment given >2x/day)
Severe)	3	Activities of daily living limited to <50% of baseline. Medical intervention often required.
Life-threatening	4	Inability to perform basic self-care functions OR severity of illness requires hospitalization

Severity of fever will also be assessed by degree

Table 3: Fever Grading System

Intensity	Grade	Defined
<100.4°F (<38.0°C)	0	Fever (oral) Temperatures ≥100.4°F, will be confirmed by repeating temperature after waiting 20 minutes
≥100.4°F – 101.4°F (≥38.0°C – 38.6°C)	1	
≥101.5°F – 102.4°F (38.7°C – 39.1°C)	2	
>102.4°F (>39.1°C)	3	

8.5 Investigator Reporting Responsibilities

8.5.1 Adverse Event Reporting

The Office of Clinical Research Policy and Regulatory Operations (OCRPRO) (DCR/NIAID/NIH) is the Sponsor for the IND filed at the U.S. FDA. AE data will be submitted to the IND Sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

In the interest of subject safety in OCRPRO-sponsored studies and to fulfill regulatory requirements, any deaths and life-threatening SAEs due to any cause that occur during the course of the study must be reported to the OCRPRO Clinical Safety Office (CSO) by the PI within 1 business day after the clinical site becomes aware of the event, and all other SAEs must be reported as soon as possible, but no later than 3 business days after site awareness. SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form and sent to the CSO by fax or email attachment.

SAEs that have not resolved by the end of the 3-month follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE case report form (CRF) and the Safety Expedited Report Form (SERF).

SAEs will be reported to:

- Sponsor, OCRPRO, CSO: Mailing address: Clinical Safety Office, 5705 Industry Lane, Frederick MD 21704, Phone 301-846-5301, Fax: 301-846-6224, Email: rchspsafety@mail.nih.gov

- Dr. Jeffrey Cohen, Phone: 301-496-5265; Email: jcohen@niaid.nih.gov

- JHSPH IRB Phone: 410-955-3193, Toll-Free: 1-888-262-3242, Fax: 410-502-0584, Email: JHSPH.irboffice@jhu.edu. JHSPH IRB will be notified according to its guidelines, and unexpected AEs that are probably, possibly, or definitely related to the vaccine and meet other JHSPH IRB reporting criteria will be reported in the manner specified by JHSPH IRB.

- The Johns Hopkins University (JHU) Institutional Biosafety Committee (IBC): Phone: 410-955-5918, Fax: 410-955-5929. The JHU IBC will be notified of all SAEs by telephone (followed by written report), email, or fax within 1 working day of notification of the SAE occurrence.

Following notification from the PI, the OCRPRO CSO, as the representative of the IND Sponsor, will report AEs that are serious, unexpected, and related (possibly, probably, or definitely) to the vaccine to the FDA within 15 calendar days of such determination. The FDA will also be notified by phone or fax of all deaths and life-threatening SAEs that are unexpected and related to the vaccine within 7 calendar days, followed by a formal Safety Report within 15 days after being informed of the event. All SAEs and non-serious AEs will be reported to the FDA at least annually in a summary format.

8.5.2 Unanticipated Problems

Non-Serious AEs that are UPs must also be reported on the SAE/UP Report Form and sent to the CSO by fax or e-mail attachment no later than 7 calendar days of PI awareness of the event. The UPs that are not AEs are not reported to the Sponsor CSO.

8.5.3 Pregnancy

All pregnancies through study day 90 will be reported on the Pregnancy Notification/Outcome Form to the CSO within 1 business day from site awareness.

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be Serious Adverse Events (SAEs). Events that meet SAE criteria during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) are reportable on the SERF.

Pertinent obstetrical information for all pregnancies will be reported to the Clinical Safety Office (CSO) via fax or email within 3 business days from site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness on a protocol-specified form.

Pregnancy events will also be reported to the JHSPH IRB.

8.5.4 Reporting Responsibilities to the JHSPH IRB

In accordance with JHSPH IRB policy, all unexpected SAEs will be reported by the PI within 10 working days of being detected; expected SAEs will be reported annually. All local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate source documents and entered in CRIMSON, followed to resolution, and reported annually to the FDA and JHSPH IRB.

8.6 Sponsor's Reporting Responsibilities

Suspected unexpected serious adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the Sponsor will be reported to FDA as IND Safety Reports.

The Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

8.7 Halting Rules

Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The PI will closely monitor study data as they become available and will make determinations regarding the presence and grading of AEs. The AEs will be evaluated with regard to the known complications associated with administration of vaccine components. If any of the following events occur, vaccinations will be suspended until the events are reviewed with the study Sponsor (OCRPRO):

1. One or more subjects experience an SAE as defined in Section 8.1.6 of this protocol that is determined to be possibly, probably, or definitely related to the vaccine, **or**
2. One or more subjects experience a hypersensitivity reaction that is probably or definitely related to the vaccine, **or**

3. Any severe clinical illness occurs that is not explained by a diagnosis that is unrelated to vaccination, **or**
4. Two or more subjects in any cohort experience any Grade 3 systemic AE that is determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol.

The IRB, the NIAID, The Gates Foundation, the FDA, or other government agencies, may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the IRB, the IND sponsor, the FDA, and other regulatory authorities may also result in suspension of further administration of vaccine at the clinical site. The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and administration of vaccine for the entire study as applicable.

The IND Sponsor, in collaboration with the PI will determine if it is safe to resume the study.

8.8 Safety Review and Communications Plan

A safety review and communications plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

8.9 Sponsor Medical Monitor

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed to provide safety oversight of this clinical study. The Sponsor Medical Monitor will be responsible for performing safety assessments as outlined in a Safety Review and Communication Plan.

The PI is responsible for ensuring that the Safety Monitor is aware of all new safety information during the course of the clinical trial.

9. DATA COLLECTION AND MONITORING

9.1 Source Documentation

Complete source documentation (laboratory test reports and/or medical records) is required for every study subject for the duration of the study. Specified data from source documentation for subjects enrolled in the study will be entered into the CRIMSON data system. The data entry is to be completed on an ongoing basis during the study. Data entered into CRIMSON shall be performed by authorized individuals. Corrections to the data system shall be tracked electronically (password protected) with time, date, individual making the correction, and what was changed. Source documentation should support the data collected in CRIMSON and must be signed and dated by the person recording and/or reviewing the data.

The PI is responsible for the accuracy, completeness, and timeliness of the data reported to the Sponsor through the CRIMSON Data System. All data entered into CRIMSON

should be reviewed by an Investigator and signed as required with written or electronic signature, as appropriate. Data reported in CRIMSON should be consistent with source documents or the discrepancies should be explained. Source documentation will be made available for review or audit by the Sponsor or designee and any applicable Federal authorities.

9.2 Study Documentation

Study-related documentation will be completed as required by the IRB, the Sponsor, and regulatory authorities. Continuing review documentation will be submitted by the PI to the IRB on the anniversary date of initial review as specified by the IRB. An annual report will be submitted by the Sponsor to the FDA within 60 days after the anniversary date that the IND for this study went into effect. The annual report will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33, and will discuss any revisions that have been made to or are planned for the protocol.

The PI will maintain adequate records of the disposition of the investigational product, including dates of receipt and disposition, administration, quantity, and use by subjects. If the study is terminated, suspended, or completed, all unused supplies of the investigational product will be destroyed by the investigational pharmacy staff.

9.3 Retention of Records

Trial-related documents will be maintained by the PI. All trial-related documents will be stored securely to maintain the confidentiality of subjects' personal and health-related information. All records pertaining to this protocol will be stored in a locked cabinet at JHU or at an offsite, locked storage facility per CIR process and regulations.

The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records are also to be maintained in compliance with IRB/Ethics Committee (EC), state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent provided by federal, state, and local law. It is the investigator's responsibility to retain copies of source documents until receipt of written notification to the contrary from the OCRPRO/DCR/NIAID. No study document should be destroyed without prior written agreement between OCRPRO/DCR/NIAID and the PI. Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator must provide written notification of such intent to OCRPRO/DCR/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written NIAID permission must be received by the site prior to destruction or relocation of research records other than as per CIR SOP.

9.4 Protocol Revisions

No revisions to this protocol will be permitted without documented approval from the Sponsor and the IRB that granted the original approval for the study. This does not apply

to changes made to reduce discomfort or avert risk to study subjects. Furthermore, in the event of a medical emergency, the PI shall perform any medical procedures that are deemed medically appropriate. The PI must notify the Sponsor of all such occurrences.

9.5 Study Monitoring

As per ICH GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the OCRPRO/DCR/NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points and prompt reporting of all SAEs; 3) to compare abstracted information (CRIMSON pulls) with individual subject's records and source documents (supporting data, laboratory specimen records, clinical notes); and 4) to ensure study subject protection, investigators' compliance with the protocol, and study record completeness and accuracy. The monitors will also inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections), FDA and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the PI (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

10. STATISTICAL CONSIDERATIONS

10.1 Description of Statistical Methods

This study is exploratory rather than confirmatory; its purpose is to estimate AE rates and patterns of immune responses rather than to test formal statistical hypotheses. Estimates will be presented with their 95% confidence intervals. Sample size was determined based on similar exploratory vaccine trials, and also based on the sizes of the previous cohorts to be recruited into the current study. We would like to enroll at least 12 people who were H2N2, H6N1 and H9N2 pLAIV recipients. In addition, we would like to enroll at least 20 negative controls.

Descriptive approaches will be used to meet the protocol objectives as stated in Section 2 of this protocol, as well as formal statistical tests as outlined below. Results will be presented in tabular format, as well as graphically where appropriate.

10.2 Sample Size Reasoning

The null hypothesis for the study is that the HA stalk antibody response after receipt of the H5N1 pISV in the pLAIV recipients (treatment cohort, T) will be the same as in the naïve (control, C) cohort. The alternative is that the recipients of the pLAIV will have a higher stalk antibody response. Based on the previous studies of H5N1 stalk response in naïve individuals (17, 18), we assume that the naïve cohort will have a 4-fold increase in

antibodies targeting the stalk after the H5N1 pISV. A total of 15 subjects in the pLAIV cohorts will allow for 80% power to detect a 2-fold higher response (8-fold increase in antibody titers), with an alpha of 0.05. 12 subjects in the pLAIV cohort will allow for 80% power to detect a 2.1-fold higher response: an 8.2-fold increase in antibody titers.

10.3 Primary Objective:

To explore the difference in the stalk antibody response against Influenza A group 1 stalks as measured by ELISA and neutralization titer after receipt of the Monovalent Influenza Subvirion Vaccine (H5N1) in previous recipients of H2N2, H6N1 and H9N2 pLAIVs compared with healthy, pLAIV-naïve individuals.

10.4 Primary Endpoint:

1. The fold rise in titer of anti-group 1 stalk-antibodies measured by ELISA and microneutralization assay (MN) using the chimeric cH6/N1 probe, in recipients of the pLAIVs (H2N2, H6N1 and H9N2) compared to the control pLAIV-naïve cohort. In addition to testing sera following administration of the H5N1 subunit vaccine, if possible, this analysis will include sera collected before and after receipt of pLAIV.

Analysis plan: Antibody titers will be converted to a log scale, and comparisons of the antibody titers will be made using a t-test or ANOVA as appropriate.
If more than 2 cohorts are being compared, appropriate post-hoc, pairwise (as applicable) comparisons will be made with appropriate alpha-adjustments if the null hypothesis is rejected.

10.5 Secondary Objective

Assess the reactogenicity of the Monovalent Influenza Subvirion Vaccine (H5N1) in previous recipients of live attenuated influenza vaccine (LAIV) compared with healthy, pLAIV-naïve individuals.
Adverse events will be assessed, graded and recorded for 28 days after vaccination. Serious Adverse Events (SAEs) will be assessed for 3 months after the immunization.

10.6 Secondary Endpoints:

- The number of vaccine-related adverse events in the recipients of the pLAIV compared to the control cohort.

10.7 Exploratory Objectives:

- To assess the biological activity of antibodies to the HA stalk region by passive transfer of serum from study subjects to mice to assess their ability to protect the mice from lethal challenge with an influenza virus
- To correlate ELISA and neutralizing stalk antibody titers with functional ability of the post vaccine serum to protect mice from lethal challenge
- To assess the breadth of the vaccine induced antibody response by using a panel of HAs that include HA stalks from a variety of group 1 and group 2.
- To assess B- cell responses to the pISV in the previous pLAIV recipients compared with naïve individuals.

- To assess the level of serum stalk antibodies after the initial pLAIV (from stored samples) and compare to the post-pISV response.
- To assess the longevity of the stalk antibody response
- To determine the titer of anti-H5 antibodies in the 2 cohorts of subjects.
- To determine the titer of antibodies directed to the HA received in the pLAIV doses.

10.8 Exploratory Endpoints:

- Difference in survival rates between mice challenged with H5N1 after passive serum from the subjects previously vaccinated with pLAIV compared to those receiving serum from the control cohort after vaccination with H5N1 pISV.
- The correlation of the serum stalk antibody titers by neutralization assay and ELISA with the ability of the sera to protect against lethal challenge.
- Measurement of cross-reactivity of antibody responses in pLAIV primed vs. naïve individuals to a variety of viruses representing multiple HA stalks.
- The percentage of memory B cells against group 1 stalk in the recipients of the pLAIV compared to the control cohort.
- The level of stalk antibodies generated by the pLAIV – tested in sera obtained shortly after receipt of pLAIV and compared to the stalk antibodies generated after receipt of the H5N1 pISV.
- HAI titer against H5 in the pLAIV primed vs. naïve individuals.
- HAI titer against H2, H6 and H9 in the pLAIV primed individuals.

11. HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research will be conducted in compliance with the protocol, GCP, and all applicable regulatory requirements.

11.1 Institutional Review Board

The PI will be responsible for obtaining IRB approval for the study. Before the start of the study, the appropriate documents (including the protocol, informed consent form, information sheets, and advertisements) will be submitted to the reviewing IRB for written approval. The PI must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above. The PI will be responsible for obtaining IRB approval of the annual continuing review throughout the duration of the study. A copy of the study approval (including approval of the informed consent form) is to be maintained in the PI's study document binder and a copy will be supplied to the Sponsor. During the study, the PI is responsible for providing the IRB with all documents subject to review (i.e., protocol amendments, informed consent form updates, advertisements, and any written information that may be provided to the subject). The PI will also provide annual reports on the study's progress to the IRB in accordance with IRB guidelines and government regulations.

11.2 Informed Consent Process

Informed consent is a process that is initiated prior to obtaining consent from a potential participant and continues throughout the individual's study participation. The volunteer's

consent for study participation will be documented on an Informed Consent Form (ICF). The ICF must be approved by the JHSPH IRB prior to use. The ICF provides detailed information about the study agent/intervention(s), study procedures, and risks. The subject will be asked to read and review the document. Study staff will engage volunteers in discussion of study aims and procedures, and the risks and possible benefits of study participation, encourage them to ask questions and verbalize their understanding of study requirements and administer a written comprehension tool. Subjects will not be rushed or coerced into study participation. Subjects may withdraw consent at any time throughout the course of the trial. The informed consent process will be documented in the subject's research chart, as required by 21 CFR 312.62. The informed consent form will be signed and personally dated by the subject and the individual who conducted the informed consent discussion. The original signed informed consent form will be retained in the subject's chart, and a signed and dated copy will be given to the subject. (For a more detailed description consent procedures, refer to the study procedures Section 6)

11.3 Participant Confidentiality

Subjects will not be identified in any publicly released reports of this study. All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. The investigator will inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number in order to maintain subject confidentiality. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only.

Subjects' study information will not be released without the written permission of the subject, except as required, these groups may include:

1. Audit and Compliance Officers and Legal Counsel
2. OHRP
3. The U.S. Food and Drug Administration (FDA) and other similar regulatory agencies
4. The study sponsor or designee
5. Study monitors, Medical Monitors
6. Department of Health and Human Services (DHHS) agencies
7. Governmental agencies to which HIV and hepatitis testing must be reported
8. The Johns Hopkins University Bloomberg School of Public Health

11.4 Risks

Risks to the subjects are associated with study procedures and immunization. Female subjects will be cautioned of the unknown risk of study vaccines to the fetus and must agree to use effective birth control methods for the duration of the study.

11.4.1 Venipuncture

Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, bleeding, infection, lightheadedness, and syncope (rarely).

11.4.2 Nasal Wash

Risks occasionally associated with nasal wash include pain or discomfort, and very rarely, epistaxis.

11.4.3 Immunization

Possible local vaccine reactions include pain, swelling, erythema, induration or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, and myalgia may also possibly occur, with some reactions moderate or severe. A placebo-controlled trial of the H5N1 inactivated vaccine (12) showed that overall, the vaccine was generally well tolerated. The most common AE following immunization was mild pain at the injection site. Tenderness was also commonly reported. Less frequently, subjects reported malaise, myalgia or headache, although those symptoms were not statistically greater in the vaccinated group compared to the placebo group. Most reported AEs were mild (84%), some were moderate, and few were severe.

It is possible that a subject may experience a reaction that is severe or even life threatening, including, but not limited to an immune/allergy mediated response.

11.5 Benefits

Subjects will not receive any direct benefit from participation in this study. It is hoped that information gained in this study will contribute to the development of a safe and effective vaccine for the prevention of pandemic influenza.

11.6 Compensation

Volunteers who are enrolled in the study will be paid $80.00 for screening and $80.00 for each follow up visit completed on time. On vaccination day, the volunteers will be compensated $125.00 because they will be required to stay in the clinic for a substantially longer period of time. Volunteers, who attend as alternates on the day of vaccination but are not enrolled, will receive $80.00 for screening and $80.00 for serving as an alternate. Enrolled subjects will receive a $250 bonus at the end of the study for complying with all study requirements and completing all study visits on time. Subjects will be compensated $80 each time they are asked to return to the CIR for an extra outpatient visit.

The ability to achieve our research goals rests on our ability to recruit and enroll healthy subjects who received H2N2, H6N1, or H9N2 in prior CIR studies. This is a highly rarefied subset of healthy individuals and as such has a high value in terms of achieving our research goals. Recalled subjects will receive an additional bonus of $250.00 upon completion of the first 28 days of study follow-up to both incentivize enrollment and completion of key study time points and acknowledge the important role they play in the study.

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APPENDICES

Appendix A: Schedule of Study Procedures

Procedure or Test	Day -60 to 0	D0	D7	D14	D28	D56	D90	D360
Informed consent	X							
Screening evaluation	X							
Pregnancy Prevention Counseling	X	X	X	X				
Complete Physical Exam	X							
Focused Physical Exam		X	X	(X)	(X)	(X)	(X)	(X)
HbSAg, HCV, HIV	X							
CBC with differential	X							
H5N1 HAI assay	X							
Urine or serum beta-HCG (women)	X	X			X		X	
Complete medical history	X							
Review of exclusion/inclusion	X	X						
Vital signs	X	X	X	X				
Administer Vaccine		X						
Interim history		X	X	X	X	X	X	X
Serology		X	X	X	X	X	X	X
Whole blood (PBMC) (mL)		X	X	X	X	X	X	X

Appendix B: Local and Systemic Adverse Event Grading Systems

These tables are modified versions of the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials to be used to grade adverse events.

Tables for Clinical Abnormalities

Table 4: Local Reaction Grading System

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Pruritus	Does not interfere with activity	Requiring po or topical treatment >24 hrs or IV meds or steroids for ≤ 24 hrs	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

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

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

Table 5: Vital Signs and Systemic Reactions Grading System

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Tachycardia - beats per minute **	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute**	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic – mm Hg)	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue/Malaise	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization

* Subject should be at rest for all vital sign measurements.

** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Appendix C: H5N1 Vaccine Product Insert

Product Insert can be found online at the following site:

https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM112836.pdf

Appendix D: H5N1 Vaccine Product label