Response to Influenza Vaccine During Pregnancy

Study Protocol and Statistical Analysis Plan

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Response to Influenza Vaccine During Pregnancy

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TABLE OF CONTENTS

1 PROTOCOL SUMMARY

Response to Influenza Vaccine During Pregnancy	
Phase: IV	
Population: Up to 50 healthy pregnant women ages 18-49 years Intervention: Women will be vaccinated with the Quadrivalent, Inactivated Influenza Vaccine (IIV4) in Year 1 at Screening/Entry with subsequent visits at Day 7 and Day 28. No more than 60 mLs of blood will be collected from volunteers at each visit. In Year 2, participants will be vaccinated with IIV4 at Day 0. 60 mLs of blood will be collected at Day 0, Day 7, and Day 28 to compare immune response to influenza vaccination between the pregnant and non-pregnant states.	
Number of Sites: 1	
Study Duration: 60 months for enrollment, study completion, analysis	
Subject Participation Duration: Approximately 56 weeks	
Description of Agent or Intervention: Quadrivalent, Inactivated Influenza Vaccine (IIV4)	
Endpoint	**Primary:** HAI titers
Description of Study Design: This is a Phase IV study of healthy pregnant women who will receive the current seasonal IIV4 during two consecutive influenza seasons (second year while non-pregnant). Immunization will be at Screening/Entry in Year 1 and Day 0 in Year 2. Blood samples for immunogenicity assays will be collected at the time of immunization and at Days 7 (6-8) and 28 (21-35) in both Year 1 and Year 2. Study Participants: Up to 50 pregnant women will be given seasonal IIV4 in two consecutive influenza seasons. Each volunteer will complete a total of 6 visits: Day 0, Day 7 and Day 28 in both Year 1 and Year 2.	
Estimated Time to Complete Enrollment: Participants will be enrolled Aug-Jan until enrollment is complete; anticipated time = 2 years	

2 ENDPOINTS

Primary: HAI Titers at Week 4 after vaccination

Secondary: Grade 2 or Higher Adverse Effects

3 BACKGROUND

Influenza virus infection represents a major cause of morbidity and mortality in the United States, resulting in 9.2-35.6 million illnesses, 140,000-710,000 hospitalizations, and 12,000-56,000 deaths annually since 2010 (1). This results in life-years lost, quality of life reduced, and an economic and medical cost of between $71 billion and $167 billion per year. Along with the fact that influenza can trigger deadly pandemics such as the 1918 pandemic that resulted in the deaths of 50 million to 100 million people (3-5% of the global population), these data illustrate the high level of priority needed to curb the public health threat of influenza. Influenza continues to be a problem due to the problems with the current vaccine strategy. Now our vaccines incorporate only three or four of the large number of circulating strains, with no adjuvant except for the elderly, and provide only 10-60% protection from influenza infection (2). Annual modifications of the vaccine are required each year, based on predictions of the next season's circulating strains. This strategy is often thwarted by the annual antigenic changes that occur in influenza virus composition. In addition to the issues with seasonal influenza, pandemic influenza typically starts with unpredicted strains, for which entirely new vaccines are required, significantly hindering our preparedness. These reasons and more highlight the necessity for a better understanding of the key components of an effective immune response and how this could lead to a universal vaccine. Pregnant women are known to have more severe influenza. In this study, we will investigate immunologic reasons to explain the increased morbidity/mortality associated with influenza in pregnant women

3.1 Potential Risks and Benefits

3.1.1 Potential Risks

This protocol will immunize adult participants with IIV4 (IM), which is recommended for use in the age groups and population studied. The discomforts of this study are those of receiving IM injection and blood drawn from an arm vein, and possible reactions to the vaccine. Drawing blood causes transient discomfort and may cause fainting. Infection at the site where blood will be drawn or where the vaccination is given is extremely unlikely but is a potential risk. Bruising at the site of blood drawing may occur, but can be prevented or lessened by applying pressure for several minutes immediately after the blood draw. Immediate allergic reactions to vaccine, including anaphylaxis, are extremely rare (approximately 1 person in 4,000,000), and might occur as a skin rash such as hives, difficulty breathing, fainting, drop in the blood pressure and death. Such reactions can usually be stopped by emergency medications administered by study personnel. Vaccine recipients may develop systemic reactions such as fever, headaches, body aches, and fatigue. These reactions are usually greatest within the first 24 to 72 hours after vaccination and last 1 to 2 days. Analgesics (*e.g.*, aspirin or Tylenol®) and rest will generally relieve or moderate these symptoms. Other hypersensitivity reactions, including Arthus reactions resulting in large local swelling reactions, are also possible. Although Guillain-Barré syndrome may have been associated with the 1976-77 inactivated swine influenza vaccine and TIV vaccines used in early 1990's, subsequent inactivated vaccines have not been associated with an increased risk of this condition.

Participation in this study may involve risks to the participant which are currently unforeseeable.

3.1.1 Potential Benefits

Participants given the seasonal influenza vaccine are likely to experience decreased frequency and severity of subsequent influenza infection. The beneficial role of influenza vaccination has been recognized increasingly over the past several years as more information has become available about the high rate of morbidity and mortality from this respiratory pathogen.

4 STUDY DESIGN

This is a phase IV study of 50 healthy 18-49 year old pregnant women who will be given licensed seasonal IIV4. There are no exclusions for ethnicity or race. Following confirmation of written informed consent, baseline blood samples will be drawn from all study participants prior to immunization.

Intervention Group: Up to 50 healthy pregnant volunteers, 18-49 year old, will be given seasonal quadrivalent inactivated influenza vaccine (IIV4). Each volunteer will complete a total of 3 visits in Year 1: Day 0 (Screening/Entry/Immunization visit), and Day 7 +/- 1 and Day 28+/- 7 and a total of 3 visits in Year 2 (when not pregnant): Day 0 (Immunization visit), and Day 7 +/- 1 and Day 28 +/- 7 (post-immunization). All visits will consist of drawing blood for study assays and monitoring for serious adverse events (SAEs) and adverse events (AEs). In Year 2, if participant is pregnant, they will not be entered into Year 2 of the protocol.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

1. 18-49 year old pregnant woman
2. Willing and able to complete the informed consent process
3. Availability for follow-up for the planned duration of the study with the expected delivery date more than 8 weeks after vaccination (i.e., before 32 weeks gestational age of pregnancy)
4. Acceptable medical history by review of inclusion/exclusion criteria

5.2 Subject Exclusion Criteria

1. Prior off-study vaccination with seasonal influenza vaccine within three months of study entry.
2. Life-threatening reactions to previous influenza vaccinations.
3. Allergy to egg or egg products or to vaccine components including gentamicin, gelatin, or arginine.
4. Active systemic or serious concurrent illness, including febrile illness on the day of vaccination.
5. Hemoglobin <9 g/dL within the past 8 weeks (those without a value within that timeframe will have a Hdb drawn at entry).
6. History of immunodeficiency (including HIV infection).
7. Known or suspected impairment of immunologic function; may include significant liver disease, diabetes mellitus treated with insulin or moderate to severe renal disease
8. Chronic Hepatitis B or C.
9. Recent or current use of immunosuppressive medication, including systemic glucocorticoids (corticosteroid nasal sprays, inhaled corticosteroids, and topical corticosteroids are permissible). Use of oral steroids (≤10mg prednisone-equivalent/day) may be acceptable after review by the Protocol Director.
10. Malignancy (other than squamous cell or basal cell skin cancer) within the past 5 years.
11. Autoimmune disease (such as Rheumatoid arthritis, lupus, etc.) treated with immunosuppressive medication such as Plaquenil, methotrexate, prednisone, Enbrel.
12. Receipt of blood or blood products within the past 6 months or planned used during the study.
13. Receipt of an inactivated vaccine 14 days prior to study enrollment, or planned vaccinations prior to completion of last annual study visit.
14. Receipt of a live, attenuated vaccine within 28 days prior to enrollment or planned vaccination during post-vaccination follow-up.
15. Need for allergy immunization (that cannot be postponed) until after the last study visit.
16. History of Guillain–Barré syndrome.
17. Use of investigational agents within 30 days prior to enrollment or planned use during the study.
18. Donation of the equivalent of a unit of blood within 4 weeks prior to enrollment or donation of platelets within 2 weeks of enrollment or planned donation prior to completion of the last visit.
19. Any condition which, in the opinion of the investigator, might interfere with volunteer safety, study objectives or the ability of the participant to understand or comply with the study protocol.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

The study product will be shipped from the manufacturer to the investigational pharmacy at the study site. IIV4 will be supplied by Glaxosmithkline Biologicals as Fluarix® Quadrivalent.

6.1.2 Formulation, Packaging, and Labeling

FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) will make annual recommendations regarding the composition of the U.S. seasonal influenza vaccines based on global influenza virus surveillance data related to epidemiology and antigenic characteristics, serological responses to trivalent and quadrivalent seasonal vaccines, and the availability of candidate strains and reagents.

The season-specific manufacturer package inserts for each study year will be attached in the IRB protocol submissions when available.

Fluarix® Quadrivalent: Each 0.5 mL dose of Fluarix Quadrivalent (IIV4) will contain a total of 60 µg (15 µg of each strain) of influenza virus hemagglutinin of each of the 4 strains selected for the seasonal formulation. The vaccine will be supplied in a prefilled, single dose syringe, 0.5 mL (no preservative), single dose vial 0.5 mL (no preservative) or multi-dose vial, 5 mL (with preservative). Each multi-dose vial contains ten 0.5 mL doses.

6.1.3 Product Storage and Stability

Fluarix® Quadrivalent vaccine presentations should be refrigerated at 2° to 8°C (35° to 46°F). Vaccine that has been frozen will be discarded. Between uses, multi-dose vials will be returned to the recommended storage conditions at 2° to 8°C (35° to 46°F). Vaccine should not be used after the expiration date shown on the label.

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

IIV4 vaccine will be administered as a 0.5 mL dose, with a sterile, disposable syringe and needle by IM injection into the deltoid muscle. The participant will choose whether the injection will be administered into the right or left deltoid.

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

The study article will be shipped by the manufacturer to the study site. Use and disposition of each unit of vaccine will be appropriately documented by the Clinical Core staff in accordance with ICH GCP. Unused products will be given to researchers.

6.4 Concomitant Medications

At entry and at each study visit, the participant will be questioned about medication use and the information will be recorded. Medication history will include vaccinations, allergy shots or PPD tests received and medications taken within 28 days prior to enrollment, as well as vaccinations, allergy shots or PPD tests received and current prescription and over-the-counter medications used throughout the study period. The investigator should be consulted regarding eligibility if the participant is taking oral steroid medications or medications for treatment of autoimmune disease (such as Plaquenil, methotrexate, prednisone, Enbrel), or any other medications which might indicate a condition that precludes participant compliance with the protocol.

7 STUDY SCHEDULE

7.1 Schedule of Events

Evaluation	Year 1			Year 2			Early Termination Visit
	D0	D7	D28	D0	D7	D28	
Written Informed Consent	X						
Eligibility Determination	X						
Demographics/Medical History/Pregnancy History	X						
Update Medical Hx/ Review Eligibility Requirements				X			
Concomitant Medications	X	X	X	X	X	X	X
Signs/Symptoms/Adverse Events	X	X	X	X	X	X	X
Vital Signs/Weight	X	If clinically indicated		X	If clinically indicated		
Height	X						
Hemoglobin*	X						
Phlebotomy	X	X	X	X	X	X	
IIV4 Administration	X†			X			

* An adequate hemoglobin level (i.e., >9.0g/dL) from within 8 weeks of entry can substitute for the research hemoglobin draw); † - IIV in Year 1 may be administered by clinic staff at Stanford Ob/Gyn Flu Clinic as part of participant's routine care (i.e., outside of research study)

7.2 Year 1, Day 0

The following will be performed at screening (see Section 7.6 for a detailed explanation of each evaluation)

- Written Informed Consent
- Eligibility Determination
- Demographics/Medical History/Pregnancy History
- Concomitant Medications
- Signs/Symptoms/Adverse Events
- Vital Signs/Weight
- Height
- Hemoglobin
- Phlebotomy
- IIV4 Administration (may be administered by clinic staff at Stanford's Ob/Gyn Clinic as part of participant's recommended obstetrical clinical care)

7.3 Year 2, Day 0 Evaluation

The following will be performed at the vaccination visit for Year 2:

- Update Medical History/Review Eligibility Requirements
- Concomitant medications
- Signs/Symptoms/Adverse Events
- Vital Signs/Weight
- Phlebotomy
- IIV4 Administration

7.4 Post-vaccination Visits

Post-vaccination visits occur following the Year 1, Day 0 Visit and the Year 2, Day 0 Visit. Week 1 visits (in both Year 1 and Year 2) occur at Day 7 (+/- 1 day) after IIV administration; Week 4 visits (in both Year 1 and Year 2) occur at Day 28 (+/- 7 days) after IIV administration.

The following will occur at each Post-vaccination Visit:

- Concomitant medications
- Signs/Symptoms/Adverse Events
- Vital Signs/Weight (only if clinically indicated)
- Phlebotomy

7.5 Early Termination Visit

The Protocol Director should be notified within 48 hours if a participant terminates the study prior to completing all study visits.

If a participant discontinues the study early for any reason then the following assessments for the Early Termination Visit should occur:

- Concomitant medications
- Signs/Symptoms/Adverse Effects

However, if the participant discontinues the study between Year 1 and Year 2, the Day 28 visit of Year 1 can serve also as the Early Termination Visit.

7.6 Definition of Evaluations

7.6.1 Written Informed Consent

Participants will be given the IRB-approved informed consent form at the Screening/Entry Visit. A discussion of risks and possible benefits of participation in this study will be provided to the participants. Participants then will be asked for any questions and to read/sign the consent form. The consent form will be signed prior to the performance of any study procedures. A copy of the informed consent document will be given to the participant for her records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

7.6.2 Eligibility Determination

At the completion of all protocol-defined screening procedures, the Eligibility Determination Form will be completed and signed by the Study Coordinator according to study criteria (see sections 4.1 and 4.2). Eligible participants giving informed consent will be enrolled in the study.

7.6.3 Demographics/Medical History/Pregnancy History

Participant demographics will include date of birth and race/ethnicity. A medical history will be obtained and must be recorded in the source documents. The medical history must include:

- All past medical diagnoses, acute or chronic
- Influenza Vaccination History within the past 5 years

A pregnancy history will be obtained and must be recorded in source documents. The pregnancy history must include:

- Expected delivery date (in order to calculate gestational age)
- Pre-pregnancy weight
- Number of prior pregnancies
- History of prior preterm births

7.6.4 Update Medical History/Review Eligibility Requirements

At the Day 0 visit of Year 2, the medical history from Year 1 will be updated. Additionally, eligibility criteria will be reviewed and only participants who still meet all Inclusion Criteria (other than the pregnancy criterion) and still meet no Exclusion Criteria will be entered into Year 2 of the study. Participants no longer eligible will be terminated from the study.

7.6.5 Concomitant Medications

At the Entry Visit record all current and past (within the last 28 days) prescription and non-prescription medications. At subsequent visits, record changes in medications since the last study visit. Additionally, record any vaccinations, allergy shots, or PPD tests received within the past 28 days.

7.6.6 Signs/Symptoms/Adverse Events

All Grade 2 or higher signs, symptoms, and toxicities and their relationship to study procedures must be documented in the participant's record.

Any sign or symptom that leads to discontinuation of study, regardless of grade, must be recorded on source documents.

Refer to the Common Terminology for Adverse Effects, Version 5.0, November 27, 2017, located at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

Report diagnoses for clinical events and other diseases and record whether they were related to study procedures.

7.6.7 Vital Signs and Weight

Assessment of vital signs will include measurement of resting blood pressure, pulse, respiratory rate, and temperature. Weight should be measured in kilograms.

7.6.8 Height

Height should be measured in centimeters.

7.6.9 Hemoglobin

If no clinical value for hemoglobin is available from clinical care within 8 weeks prior to entry, a hemoglobin will be assessed at the Screening/entry visit.

7.6.9 Phlebotomy

Phlebotomy will be performed at each visit using antiseptic technique. See Appendix 1: Blood Draw Requirements for blood draw instructions for each visit.

7.6.10 IIV4 Administration

Participants will be administered the seasonal IIV4 vaccine in the left or right deltoid muscle using antiseptic technique. Volunteers will be observed in clinic for 15 minutes after vaccination to monitor for any immediate serious reactions. The location of the injection, lot number and expiration date will be documented.

In Year 1 for participant convenience, the IIV4 may be given as part of participant's routine clinical care (i.e., outside of this research protocol) as part of participant's recommended obstetrical clinical care. In this case, the administration of the vaccine will be done on the same day following the Day 0 evaluations described in Section 7.1

In Year 2, all study related procedures, including the administration of the seasonal IIV4 vaccine will be completed by the Research Study Team as part of this study protocol.

8 ADVERSE EVENTS AND TOXICITY MANAGEMENT

8.1 Definitions of AEs, Adverse Reactions, and Serious Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study participant administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. AEs may also include pre-or post-treatment complications that occur as a result of protocol specified procedures, lack of efficacy, overdose, drug abuse/misuse reports, or occupational exposure. Preexisting events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs. An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an adverse event and must be reported
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the Screening Visit that do not worsen
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae
- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented as medical history.

A serious adverse event (SAE) is defined as an event that results in the following:

- Death
- Life-threatening (note: The term “life-threatening” in the definition of “serious” refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- A medically important event or reaction; such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is reportable under expedited reporting rules. Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and be subject to expedited reporting requirements.

Clinical laboratory abnormalities and other abnormal assessments without clinical significance are not recorded as AEs or SAEs. However, laboratory abnormalities that require medical or surgical intervention or lead to study drug discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (e.g., electrocardiogram, x-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE as described above. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (e.g., anemia), not the laboratory result (i.e., decreased hemoglobin).

8.2 Assessment of Adverse Events and Serious Adverse Event

The Protocol Director is responsible for assessing AEs and SAEs for causality and severity, and for final review and confirmation of accuracy of event information and assessments.

The Protocol Director is responsible for assessing the relationship to study drug treatment using clinical judgment and the following considerations:

- No: Evidence exists that the adverse event has an etiology other than the study drug. For SAEs, an alternative causality must be provided (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

- Yes: There is reasonable possibility that the event may have been caused by the investigational medicinal product.

Ineffective treatment should not be considered as causally related in the context of adverse event reporting. The relationship to study procedures (e.g., invasive procedures such as venipuncture or biopsy) should be assessed using the following considerations:

- No: Evidence exists that the adverse event has an etiology other than the study procedure.

- Yes: The adverse event occurred as a result of protocol procedures (e.g., venipuncture).

The severity grading of AEs will be assessed as Grade 1, 2, 3, or 4 using the Common Terminology for Adverse Effects, Version 5.0, November 27, 2017, located at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf and Grade 2 or higher AEs recorded.

For AEs associated with laboratory abnormalities, the event should be graded on the basis of the clinical severity in the context of the underlying conditions; this may or may not be in agreement with the grading of the laboratory abnormality.

8.3 Instructions for Reporting Adverse Events and Serious Adverse Events

SAEs, regardless of cause or relationship, that occur after the participant first consents to participate in the study (i.e., signing the informed consent) and throughout the duration of the study, including the protocol-required post treatment follow-up period, must be reported on the CRF. This also includes any SAEs resulting from protocol-associated procedures performed from screening onwards. All AEs, regardless of cause or relationship, that occur from initiation of study drug until 4 weeks after last administration of study drug must be reported on the CRF. Any SAEs and deaths that occur after the Post-Treatment follow-up visit OR within 30 days of the last dose of study drug (whichever is longer), regardless of causality, should also be reported. All AEs should be followed up until resolution or until the adverse event is stable, if possible. Investigators are not obligated to actively seek SAEs after the 30-day period.

8.4 Reporting Procedures

8.4.1 Regulatory Reporting for Studies Not Conducted Under an IND

For those events meeting the previously described definition of Serious Adverse Events, the completion of an SAE report form is required. For SAEs related to vaccine, a VAERS form will be filled out and submitted to the Vaccine Adverse Event Reporting System (VAERS) per federal regulations. The VAERS form will simultaneously be sent to the sponsor. SAEs and events that meet the Prompt Reporting guidelines (events meeting the criteria for Unanticipated Problems) will also be reported to the Stanford IRB as required. Unexpected deaths or life-threatening experiences related to the research will be reported to the sponsor and to the IRB within 5 working days from when the investigator learns of event. SAEs not related to vaccine will be reported to the IRB on an annual basis.

8.5 Safety Oversight

The Clinical Core Principal Investigator will oversee compliance with the protocol, the participant's safety and any unanticipated problems involving risks to participants and will report these events as described above. Unanticipated problems and serious adverse events will be reported to the Stanford IRB as required.

9 CRITERIA FOR STUDY DISCONTINUATION

9.1 Criteria for Permanent Study Discontinuation

- Requirement for prohibited concomitant medications (such as immunosuppressant medication)
- Pregnancy during Year 2 of Study
- Participant no longer meeting eligibility requirement at Day 0 visit of Year 2 (other than pregnancy requirement)
- Clinical reasons such that continued participation in the study may be detrimental to the participant's health as judged by the Protocol Director
- Failure by the participant without reasonable cause to attend study visits
- Request by the participant to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- Participant died.
- At the discretion of the IRB or Study Investigators.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

We hypothesize that exposure to influenza vaccination will result in immunity to influenza in the majority of study participants and will be well-tolerated forming the basis of novel immunologic insights.

10.2 Sample Size Considerations

The sample size was selected based on feasibility.

10.3 Final Analysis Plan

We will report the percentage of participants having a 4 fold increase in HAI titers at Week 4 in Year 0 and Year 1 and the participants experiencing Grade 2 or higher AEs.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All participant information will be obtained by the investigators and their support staff and will remain confidential. Specimens for research laboratory testing will be coded by a unique participant ID number and data for individual participants will be coded for data analysis. A database containing a code key will be kept on a computer that is password secured or a locked cabinets and only available to clinical research staff. Participants will not be identified in any reports or publications that may result from the study. Personal identifierswill be removed for analyses and publications.

Participant confidentiality is held strictly in trust by the participating investigators, their staff, and their agents. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating volunteers. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The clinical study site will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives.

Quality Control and Quality Assurance

Quality Control and Quality Assurance activities will generally be completed as outlined in the current version of the Stanford Quality Management Plan. Chart audits for the trial will be conducted for research participant records utilizing the SLVP Chart Audit Tool. Audits will be conducted on a random sampling of participant charts in accordance with the low risk to volunteers participating in the trial. Charts will be randomly selected from among those not previously audited. Results of these audits will be summarized using the SLVP QM_QA Summary Report tool, and shared with research staff at staff meetings, as necessary.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

The Protocol Director will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

12.2 Institutional Review Board

Prior to enrollment of participants into this trial, the protocol and the informed consent form will be reviewed and approved by the appropriate IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DAIT. Notification of the IRB's composition and the institutions Federal Wide Assurance number will be provided to DAIT as needed. Should amendments to the protocol be required, the amendments will be reviewed by the sponsor and/or the investigators and submitted to the IRB. Volunteers will be compensated for their participation in this study. Compensation will be in accordance with the local IRB’s policies and procedures and requires IRB approval.

12.3 Subject Confidentiality

All participant information will be obtained by the investigators and their support staff and will remain confidential. Specimens for laboratory testing will be coded by participant number and data for individual participants will be coded for data analysis. A database containing a code key will be kept on a computer that is password secured and available only to study staff. Participants will not be identified (except for age) in any reports or publications that may result from the study. Personal identifiers, except for age, will be removed for publications. Upon completion of the study, data containing PHI will be retained for 50 years from the time of informed consent.

Participant confidentiality is held strictly in trust by the participating investigators, their staff, and their agents. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating volunteers.
The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The clinical study site will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study.

12.4 Future Use of Stored Specimens

After the study is complete, residual specimens will be stored for future research. As new scientific discoveries identify technologies or mediators that might be useful in studying the immune response, stored samples may be used to explore new information that could be made available with these advanced methods. Volunteer specimens will be stored under a unique identifier. The volunteer's name or other personal identifiers will not be available in any data shared with outside investigators. New studies using stored samples will be reviewed by the IRB as required.

13 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents and data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or dark blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed out with a single line, and initialed and dated to indicate the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Data entered into the data entry system that is derived from source documents or data collection forms should be consistent with the source documents and data collection forms or the discrepancies should be explained.

13.1 Data Management Responsibilities

All source documents will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Serious adverse events will be assessed for causality, and reviewed by the site PI or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The PI (or designee) will be responsible for data management, quality review, analysis, and reporting of the study data.

13.2 Data Capture Methods

Clinical data (including SAEs) will be entered into a 21 CFR Part 11-compliant data entry system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or data collection forms.

13.3 Types of Data

Data for this study will include clinical, safety and outcome measures.

13.4 Timing/Reports

Data will be reviewed on an ongoing basis according to the site Quality Management Plan. Data analysis will begin once all clinical data have been collected and verified for accuracy. Preliminary analyses of outcome measures will begin as soon as laboratory data are available. Participants will receive a unique study ID at enrollment. All data for study analysis will be coded by study ID number and will be password-protected. Coded

protected health information will be provided only as needed for data analysis of study outcome measures. Personal identifierswill be removed for publications.

Protected health information may be disclosed as requested by The Office for Human Research Protections in the U.S. Department of Health and Human Services, the sponsor, or Stanford University Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary.

13.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including CRFs, source documents, data collection forms, consent forms, and medication inventory records, must be retained by the investigator for at least 3 years and in accordance with Stanford University and Stanford IRB requirements and until the sponsor authorizes transfer or destruction of study records.

13.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or Good Clinical Practice (GCP). The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations. All deviations from the Protocol will be addressed in a study subject data collection form. Protocol deviations will be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14 PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. Any clinical trial starting enrollment after 27SEP2007 must be registered either on or before the onset of participant enrollment.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (*e.g.*, Phase 1 trials), would be exempt from this policy.

15 REFERENCES

1. Osterholm MT, Kelley NS, Sommer A, Belongia EA: Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis, Lancet Infect Dis 2012, 12:36-44

2. Goodwin K, Viboud C, Simonsen L: Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine. 2006 Feb 20;24(8):1159-69.

16 APPENDIX 1: BLOOD DRAW INSTRUCTIONS FOR RESPONSE TO INFLUENZA VACCINE DURING PREGNANCY (IRB-50163)

IRB approved 60 mL at all visits; all blood draws will occur at CTRU and all samples will be sent to the HIMC for processing

Visit	Samples	CTRU Procession Instructions	Volume Collected
Year 1 Visit 1 (Day 0)	1 x 10 mL Serum Red Top 1 x 2.5mL PAXgene Tube 4 x 10mL Heparin Green Top 1 x 4mL Heparin Green Top 1 X 2 mL Lavender top (if required for a CBC)	Sera Aliquot: approximately 10 aliquots of 0.5mL/vial of the serum from 10mL of blood. PAXgene saved for future testing; Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial; Lavender top should be sent to clinical lab for Hemoglobin	56.5 mL – 58.5 mL (depending if Hgb required at entry)
All other visits (Year 1 visits 2 and 3; Year 2 visits 1, 2, and 3)	1 x 10 mL Serum Red Top 5 x 10mL Heparin Green Top	Sera Aliquot: approximately 10 aliquots of 0.5mL/vial of the serum from 10mL of blood. Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	60 mL