

# The Effects of Attenuated Versus Inactivated Flu Vaccine in Twin Sets

Study Protocol and Statistical Analysis Plan

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**The Effects of Attenuated Versus Inactivated Flu Vaccine in Twin Sets**

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## 1 PROTOCOL SUMMARY

<b>Title: The Effects of Attenuated Versus Inactivated Flu Vaccine in Twin Sets</b>	
<b>Phase:</b> IV	
<b>Population:</b> Up to 40 healthy children and adult twins (40 total twin pairs), ages 2-20 years, will be randomized to either Group A or Group B (1 twin pair to group A and 1 twin pair to Group B):  <u>Group A:</u> Up to 10 monozygotic (MZ) and 10 dizygotic (DZ) twin volunteers will be given seasonal quadrivalent inactivated influenza vaccine (IIV4).  <u>Group B:</u> Up to 10 MZ and 10 DZ twin volunteers will be given quadrivalent live, attenuated influenza vaccine (LAIV4).	
<b>Number of Sites:</b> 1	
<b>Study Duration:</b> 60 months for enrollment (48 months for enrollment), study completion, analysis	
<b>Subject Participation Duration:</b> 4-5 weeks	
<b>Description of Agent or Intervention:</b> <b>Quadrivalent, Inactivated Influenza Vaccine (IIV4)</b> <b>Quadrivalent, Live Attenuated Influenza Vaccine (LAIV4)</b>	
<b>Objectives:</b>	<b>Primary:</b> Determine the proportion of participants experiencing Grade 2 or Higher Adverse Effects
	<b>Secondary:</b> Determine HAI Titers at Week 4 after vaccination
<b>Description of Study Design:</b> This is a Phase IV study of healthy monozygotic and dizygotic children and adults who will receive the current seasonal influenza vaccine. Twin pairs will be randomized to either Group A or Group B (one twin to Group A and one twin to Group B). There are no exclusions for gender, ethnicity or race. The volunteers will be enrolled into one of 2 groups over a 4-year period. Immunization will be at Day 0, blood samples for immunogenicity assays will be collected prior to immunization and at Days 7 (6-8) and 28 (21-35).	
<u>Group A:</u> Up to 10 monozygotic (MZ) and 10 dizygotic (DZ) twin volunteers, 2-20 years old, will be given seasonal quadrivalent inactivated influenza vaccine (IIV4). Each volunteer will complete a total of 3 visits: Day 0, Day 7 and Day 28.	
<u>Group B:</u> Up to 10 MZ and 10 DZ twin volunteers, 2-20 years old will be given seasonal quadrivalent live, attenuated influenza vaccine (LAIV4). Each volunteer will complete a total of 3 visits: Day 0, Day 7 and Day 28.	
<b>Estimated Time to Complete Enrollment:</b> Participants will be enrolled Aug-Feb in each of 4 years	

## 2 OBJECTIVES

**Primary:** Determine the proportion of participants experiencing Grade 2 or Higher Adverse Effects

**Secondary:** Determine HAI Titers at Week 4 after vaccination

### **3 BACKGROUND AND RATIONALE**

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. In study, we will use twins to investigate the role of genetics in immune response to both inactivated influenza vaccine (IIV4) and live attenuated inactivated vaccine (LAIV4).

#### **3.1 Potential Risks and Benefits**

##### **3.1.1 Potential Risks**

This protocol will immunize children and adult participants with either IIV4 (intramuscular) or LAIV4 (intranasal) vaccines that are licensed and recommended for use in the age groups studied. The discomforts of this study are those of receiving IM injection or intranasal application of the vaccine, 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 in general 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. LAIV4 has the potential for transmission of influenza to close contacts with severely weakened immune systems (requiring care in a protected environment, such as a bone marrow transplant unit). It is not known whether

LAI4 (FluMist Quadrivalent) is excreted in human milk. Because some viruses are excreted in human milk, lactating women will not be eligible for this study.

### **3.1.2 Known 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 40 healthy 2-20 year old volunteers (all members of twin sets) who are given licensed seasonal influenza vaccine. Following confirmation of written informed consent (and assent, if applicable), baseline blood samples will be drawn from all study participants prior to immunization. The volunteers will be enrolled into one of 2 groups (randomized by twin pair):

Group A: Up to 10 healthy MZ and 10 healthy DZ twin volunteers, 2-20 year old, will be given seasonal quadrivalent inactivated influenza vaccine (IIV4). Each volunteer will complete a total of 3 visits: Day 0 (pre-immunization), and Day 7 +/- 1 and Day 28 +/- 7 (post-immunization). All visits will consist of drawing blood for study assays and monitoring for adverse events (SAEs) and adverse events (AEs).

Group B: Up to 10 healthy MZ and 10 healthy DZ twin volunteers, 2-20 year old, will be given quadrivalent live, attenuated influenza vaccine (LAIV4). Each volunteer will complete a total of 3 visits: Day 0 (pre-immunization), 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).

## 5 STUDY ENROLLMENT AND WITHDRAWAL

### 5.1 Subject Inclusion Criteria

1. 2-20 years old with a weight of  $\geq 10.0$  kilograms
2. Member of a monozygotic (MZ) or dizygotic (DZ) twin set
3. Willing to complete the informed consent process (and assent for minors 2-17 years of age)
4. Twin pair also agreeable and eligible for study inclusion
5. Availability for follow-up for the planned duration of the study (at least 28 days after immunization)

### 5.2 Subject Exclusion Criteria

1. Prior off-study vaccination with seasonal influenza vaccine within three months of study vaccination
2. Life-threatening reactions to previous influenza vaccinations
3. Asthma
4. Allergy to egg or egg products or to vaccine components including gentamicin, gelatin, arginine or MSG.
5. Active systemic or serious concurrent illness, including febrile illness on the day of vaccination
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. Hospitalization in the past year for congestive heart failure or emphysema.
9. Chronic Hepatitis B or C.
10. Recent or current use of immunosuppressive medication, including systemic glucocorticoids (corticosteroid nasal sprays and topical steroids are permissible). Use of oral steroids ( $\leq 10$ mg prednisone-equivalent/day) may be acceptable after review by the investigator.
11. Participants in close contact with anyone who has a severely weakened immune system
12. Malignancy (other than squamous cell or basal cell skin cancer) within the past 5 years
13. Autoimmune disease (such as rheumatoid arthritis, lupus, etc.) treated with immunosuppressive medication such as plaquenil, methotrexate, prednisone, Enbrel
14. History of blood dyscrasias, renal disease, or hemoglobinopathies requiring regular medical follow up or hospitalization during the preceding year
15. Receipt of an influenza antiviral medication within 48 hours prior to study vaccination [If yes, may not eligible if unable to schedule at an appropriate interval].
16. Receipt of blood or blood products within the past 6 months or planned used during the study.
17. Receipt of an inactivated vaccine 14 days prior to study enrollment, or planned vaccinations prior to completion of last study visit (~ 28 days after study vaccination)
18. Receipt of a live, attenuated vaccine within 28 days prior to enrollment or planned vaccination during post-vaccination follow-up (~ 28 days after study vaccination)
19. Need for allergy immunization (that cannot be postponed) until after the last study visit.
20. History of Guillain–Barré syndrome

21. Pregnant or breastfeeding woman
22. Use of investigational agents within 30 days prior to enrollment or planned use during the study.
23. Donation of the equivalent of a unit of blood within 6 weeks prior to enrollment or donation of platelets within 2 weeks of enrollment or planned donation prior to completion of the last visit.
24. 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.

## **5.3 Treatment Assignment Procedures**

### **5.3.1 Randomization Procedures**

Individuals within each pair of twins will be randomized such that one receives IIV4 (Group A) and the other LAIV4 (Group B). Randomization will follow a permuted block design, where each twin pair is a block. Random assignment within each pair will be done by computer. Study Intervention/Investigational Product

## **5.4 Study Product Description**

### **6.1.1 Acquisition**

The study product will be shipped from the manufacturer to the study site. The quadrivalent IIV4 will be supplied by Sanofi-Pasteur as Fluzone® Quadrivalent. The LAIV4 will be supplied by MedImmune as FluMist® Quadrivalent Influenza Vaccine Live, Nasal Spray.

### **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.

Fluzone® Quadrivalent: Each 0.5 mL dose of Fluzone 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.

FluMist® (Influenza Virus Vaccine) Live, Intranasal Spray.

Each pre-filled refrigerated FluMist® Quadrivalent sprayer contains a single 0.2 mL dose. Each 0.2 mL dose contains  $10^{6.5-7.5}$  FFU (fluorescent focus units) of live attenuated influenza virus reassortants of each of the selected strains. Each 0.2 mL dose may also contain 0.188 mg/dose monosodium glutamate, 2.00 mg/dose hydrolyzed porcine gelatin, 2.42 mg/dose arginine, 13.68 mg/dose sucrose, 2.26 mg/dose dibasic potassium phosphate, and 0.96 mg/dose monobasic potassium phosphate. Each dose contains residual amounts of ovalbumin (< 0.24 mcg/dose), and may also contain residual amounts of gentamicin sulfate (< 0.015 mcg/mL), and ethylenediaminetetraacetic acid (EDTA) (< 0.37 mcg/dose). FluMist® Quadrivalent contains no preservatives. It is supplied in a package of 10 pre-filled, single-dose (0.2 mL) intranasal sprayers. The single-use intranasal sprayer contains no natural rubber latex.

#### **6.1.2 Product Storage and Stability**

Fluzone® 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.

FluMist® Quadrivalent should be stored in a refrigerator between 2 to 8°C (35-46°F) upon receipt and until use. The product must be used before the expiration date on the sprayer label and must not be frozen. The cold chain (2 to 8°C) must be maintained when transporting FluMist® Quadrivalent. Once FluMist® Quadrivalent has been administered; the sprayer should be disposed of according to the standard procedures for medical waste (e.g., sharps container or biohazard container). Vaccine should not be used after the expiration date shown on the label.

### **5.5 Dosage, Preparation and Administration of Study Intervention/Investigational Product**

For children between 2-3 years, IIV4 vaccine will be administered as a 0.25 mL dose, with a sterile, disposable syringe and needle by IM injection into the vastus lateralis muscle depending on the development of the deltoid. For participants 3 years or older, 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/guardian will choose whether the injection will be administered into the right or left deltoid.

LAIV4 vaccine dosage is 0.2 mL. Vaccine will be administered as an intranasal spray. Each sprayer contains a single dose of FluMist® Quadrivalent; approximately one-half of the contents should be administered into each nostril. 0.1 mL (i.e., half of the dose from a single FluMist sprayer) is administered into each nostril while the recipient is in an upright position. Insert the tip of the sprayer just inside the nose and rapidly depress the plunger until the dose-divider clip stops the plunger. The dose divider clip is removed from the sprayer to administer the second half of the dose (0.1 mL) into the other nostril.

## **5.6 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.

## **5.7 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.

## 6 STUDY SCHEDULE

### 6.1 Schedule of Events

	Day 0	Day 7	Day 28	Early Termination Visit
Written Informed Consent/Assent	X			
Eligibility Determination	X			
Randomization	X			
Demographics/Medical History	X			
Concomitant Medications	X	X	X	X
Signs/Symptoms/Adverse Events	X	X	X	X
Vital Signs/Weight/Height	X		If clinically indicated	
Phlebotomy	X	X	X	
IIV4 or LAIV4 Administration	X			

## **6.2 Day 0**

The following will be performed at Screening/Entry (see Section 7.6 for a detailed explanation of each evaluation):

- Written Informed Consent
- Eligibility Determination
- Randomization
- Medical History
- Concomitant Medications
- Signs/Symptoms/Adverse Events
- Vital Signs/Weight/Height
- Phlebotomy
- IIV4 or LAIV Administration

## **6.3 Post-vaccination Visits**

Post-vaccination visits occur following the Screening/Entry Visit and occur at Day 7 (+/- 1 day) and Day 28 (+/- 7 days) after IIV or LAIV administration.

The following will occur at each Post-vaccination Visit:

- Concomitant medications
- Signs/Symptoms/Adverse Events
- Vital Signs, if clinically indicated
- Phlebotomy

## **6.4 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
- Vital Signs, if clinically indicated

## **6.5 Definition of Evaluations**

### **7.6.1 Written Informed Consent**

Participants will be given the IRB-approved informed consent form (or assent form for minors participating in the study) at the Day 0 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 (and assent, if applicable) form will be signed prior to the performance of any study procedures. A copy of the informed consent (and assent, if applicable) document will be given to the participant for his/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.

#### **6.6.2 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 (and assent, if applicable) will be enrolled in the study.

#### **6.6.3 Randomization**

After a twin-set are deemed eligible, individuals within each pair of twins will be randomized based on a computer code with the older twin being given the first assignment generated by computer and the younger twin being given the other assignment.

#### **6.6.4 Demographics/Medical History**

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

- All active medical diagnoses (acute or chronic); each medication on participant's medication list should be assigned to a medical diagnosis
- History of tonsillectomy or splenectomy, allergies, or autoimmune disease
- Influenza Vaccination History over the past 5 years

#### **6.6.5 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.

#### **6.6.6 Signs/Symptoms/Adverse Events**

All Grade 2 or higher signs, symptoms, and toxicities 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](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.

#### **6.6.7 Vital Signs/Weight/Height**

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

#### **6.6.8 Phlebotomy**

Phlebotomy will be performed at each visit using antiseptic technique. See Appendix 1: Blood Draw Requirements for blood draw instructions for each visit. Maximal amount for the blood draw will be based on weight and will not exceed 3% of total blood volume in a 24-hour period consistent with the World Health Organization guidance. (3)

#### **6.6.9 IIV4 or LAIV Administration**

Participants will be administered the seasonal IIV4 vaccine in the left or right deltoid muscle using antiseptic technique or into the vastus lateralis (right or left) for participants younger than 3 or for those with limited deltoid muscle development. Participants under the age of 3 years will receive 0.25 mL and all other participants will receive the 0.5 mL dose.

LAIV4 vaccine dosage is 0.2 mL. Vaccine will be administered as an intranasal spray. Each sprayer contains a single dose of FluMist® Quadrivalent; approximately one-half of the contents should be administered into each nostril. 0.1 mL (i.e., half of the dose from a single FluMist sprayer) is administered into each nostril while the recipient is in an upright position. Insert the tip of the sprayer just inside the nose and rapidly depress the plunger until the dose-divider clip stops the plunger. The dose divider clip is removed from the sprayer to administer the second half of the dose (0.1 mL) into the other nostril.

Volunteers will be observed in clinic for 15 minutes after vaccination to monitor for any immediate serious reactions.

## 7 ADVERSE EVENTS AND TOXICITY MANAGEMENT

### 7.1 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).

## 7.2 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](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.

## **7.3 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.

## **7.4 Reporting Procedures**

### **7.4.1 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.

## **7.5 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.

## **8 CRITERIA FOR STUDY DISCONTINUATION**

### **8.1 Criteria for Permanent Study Discontinuation**

- Requirement for prohibited concomitant medications (such as immunosuppressant medication)
- 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.

## **9 STATISTICAL CONSIDERATIONS**

### **9.1 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.

### **9.2 Sample Size Considerations**

The sample size was selected based on feasibility.

### **9.3 Final Analysis Plan**

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

## **10 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 identifiers will 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, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study.

## **11 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

The Effects of Attenuated Versus Inactivated Flu Vaccine in Twin Sets. V1\_13AUG2019

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 identifiers will 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](https://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.
3. Howie SR. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ. 2011 Jan 1;89(1):46-53.

## **16 APPENDIX 1: BLOOD DRAW INSTRUCTIONS FOR THE EFFECTS OF ATTENUATED VERSUS INACTIVATED FLU VACCINE IN TWIN SETS (IRB-50200)**

IRB approved 60 mL at all visits for individuals >30 kg; all blood draws will occur at CTRU and all samples will be sent to the HIMC for processing

### **Blood Draw Instructions for individuals 10.0- <15.0 kilograms**

**(Max allowable volume to be collected = 23 mL)**

Visit	Samples	CTRU Processing Instructions	Volume Collected
Visit 1	1 x 5 mL Serum Red Top 1 x 2.5 mL PAXgene Tube 1 x 10 mL Heparin Green Top 1 x 4 mL Heparin Green Top	Sera Aliquot: approximately 5 aliquots of 0.5mL/vial of the serum from 4 mL of blood. PAXgene saved for future testing; Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	21.5 mL
Visits 2 and 3	1 x 5 mL Serum Red Top 1 x 10 mL Heparin Green Top 2 X 4 mL Heparin Green Top	Sera Aliquot: approximately 5 aliquots of 0.5mL/vial of the serum from 4 mL of blood. Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	23 mL

**Blood Draw Instructions for individuals 15.0- <20.0 kilograms**  
**(Max allowable volume to be collected = 33 mL)**

Visit	Samples	CTRU Processing Instructions	Volume Collected
Visit 1	1 x 5 mL Serum Red Top 1 x 2.5 mL PAXgene Tube 2 x 10 mL Heparin Green Top 1 x 4 mL Heparin Green Top	Sera Aliquot: approximately 5 aliquots of 0.5mL/vial of the serum from 4 mL of blood. PAXgene saved for future testing; Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	31.5 mL
Visits 2 and 3	1 x 5 mL Serum Red Top 2 x 10 mL Heparin Green Top 2 X 4 mL Heparin Green Top	Sera Aliquot: approximately 5 aliquots of 0.5mL/vial of the serum from 4 mL of blood. Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	33 mL

**Blood Draw Instructions for individuals 20.0- <25.0 kilograms**  
**(Max allowable volume to be collected = 44 mL)**

Visit	Samples	CTRU Processing Instructions	Volume Collected
Visit 1	1 x 10 mL Serum Red Top 1 x 2.5 mL PAXgene Tube 3 x 10 mL Heparin Green Top	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	42.5 mL
Visits 2 and 3	1 x 10 mL Serum Red Top 3 x 10 mL Heparin Green Top 1 X 4 mL Heparin Green Top	Sera Aliquot: approximately 4 aliquots of 0.5mL/vial of the serum from 4 mL of blood. Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	44 mL

**Blood Draw Instructions for individuals 25.0- <30.0 kilograms**  
**(Max allowable volume to be collected = 54 mL)**

Visit	Samples	CTRU Processing Instructions	Volume Collected
Visit 1	1 x 10 mL Serum Red Top 1 x 2.5 mL PAXgene Tube 4 x 10 mL Heparin Green Top	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	52.5 mL
Visits 2 and 3	1 x 10 mL Serum Red Top 4 x 10 mL Heparin Green Top 1 X 4 mL Heparin Green Top	Sera Aliquot: approximately 4 aliquots of 0.5mL/vial of the serum from 4 mL of blood. Plasma should be saved, and the PBMC processed and cryopreserved at 5-10 million cells/vial	54 mL

**Blood Draw Instructions for individuals  $\geq 30.0$  kilograms  
(Max allowable volume to be collected per IRB = 60 mL)**

Visit	Samples	CTRU Procession Instructions	Volume Collected
Visit 1	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	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	56.5 mL
Visits 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