Innate and Acquired Immunity to Influenza Infection and Immunization

NCT03028974

August 7, 2015

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Adaptive and Innate Immunity, Memory and Repertoire
in Vaccination and Infection

(U19 CCHI Renewal 2014-2019)

Project 2: Innate and Acquired Immunity to Influenza Infection and Immunization

DAIT Protocol Number: NA

DAIT Funding Mechanism: 2U19AI057229-11

Other Identifying Numbers: SLVP029; IRB Protocol: 31136

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Draft or Version Number: 2.0
Day Month Year
07AUG2015

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

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Signed: _____ Date: _____

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Title: Professor of Pediatrics, Principal Investigator

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List of Abbreviations

AE	Adverse Event/Adverse Experience
ACIP	The CDC Advisory Committee on Immunization Practices
CDC	The Centers for Disease Control
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
DAIT	Division of Allergy, Immunology and Transplantation
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
DZ	Dizygotic twin
eCRF	Electronic Case Report Form
FACS	Fluorescence Activated Cell Sorting
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IIV4	Quadrivalent Inactivated Influenza Vaccine
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
LAIV	Trivalent Live, Attenuated Influenza Vaccine
LAIV4	Quadrivalent Live, Attenuated Influenza Vaccine
MedDRA ®	Medical Dictionary for Regulatory Activities
MZ	Monozygotic twin
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NK cells	Natural Killer Cells
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections

OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SLVP	Stanford-LPCH Vaccine Program
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TIV	Trivalent inactivated influenza vaccine
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

Title: Project 2: Innate and Acquired Immunity to Influenza Infection and Immunization	
Number of Sites: 1	
Study Duration: 5 years for enrollment, vaccination, analysis	
Subject Participation Duration: 4-5 weeks	
Description of Agent or Intervention: **Quadrivalent, Live Attenuated Influenza Vaccine (LAIV4)** **Quadrivalent, Inactivated Influenza Vaccine (IIV4)**	
Objectives:	**Primary:** 1. To compare the nasal mucosal transcriptional responses to LAIV4 between the following age groups: 2-4 year old children, 9-13 year old children, and 18-49 year old adults 2. To compare the B cell response to LAIV4 between the following age groups: 2-4 year old children, 9-13 year old children, and 18-49 year old adults 3. To investigate the evolution of B cell repertoire in young children after repeated LAIV4 immunization 4. To compare the B cell response to IIV between the following age groups: 6-23 month old children, 9-13 year old children, and 18-49 year old adults 5. To investigate the evolution of B cell repertoire in young children first immunized with IIV followed by repeated LAIV4 immunization. **Secondary:** 1. To compare the B cell repertoire in response to LAIV4 versus IIV in different age groups.

Description of Study Design: This is a Phase IV study of healthy children and adults who will receive the current seasonal influenza vaccine. There are no exclusions for gender, ethnicity or race. The volunteers will be enrolled into one of 7 groups over a 5-year period. Immunization will be at Day 0. Blood and NP swab samples will be collected at various time points based upon group assignment for various immunological assays.

Group A (LAIV4/annual return): Up to six 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for all children irrespective of those who require 1 dose (prior flu vaccination history) or 2 doses (vaccine-naïve). For children requiring 2 doses of vaccine, a second immunization will be given at Day 28-32 after Dose 1. All participants in this group will be asked to return annually for repeat immunization per ACIP guidelines and blood sample collection.

Group B (LAIV4/single year): Up to twenty 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for all children irrespective of those who require 1 dose (prior flu vaccination history) or 2 doses (vaccine-naïve). For children requiring 2 doses of vaccine, a second immunization will be given at Day 28-32 after Dose 1.

Group C (LAIV4/NP swab group/single year): Up to twenty 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). NP swabs will be collected at Day 1 and Day 20-22 post-Dose 1 for all children irrespective of those who require 1 dose or 2 doses of vaccine. For children requiring 2 doses of vaccine, a second immunization will be given at least 28 days after Dose 1. No blood will be collected for this group.

Group D (IIV4/annual return): Up to six 6 month-23 month old (inclusive) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for children requiring 1 dose of vaccine (prior flu vaccination history). For children requiring 2 doses of vaccine (vaccine-naïve), a second immunization will be given at Day 28-32 after Dose 1. The second blood sample will be collected on Day 6-8 post-Dose 2. Volunteers will be asked to return annually for repeat immunization per ACIP guidelines and blood sample collection.

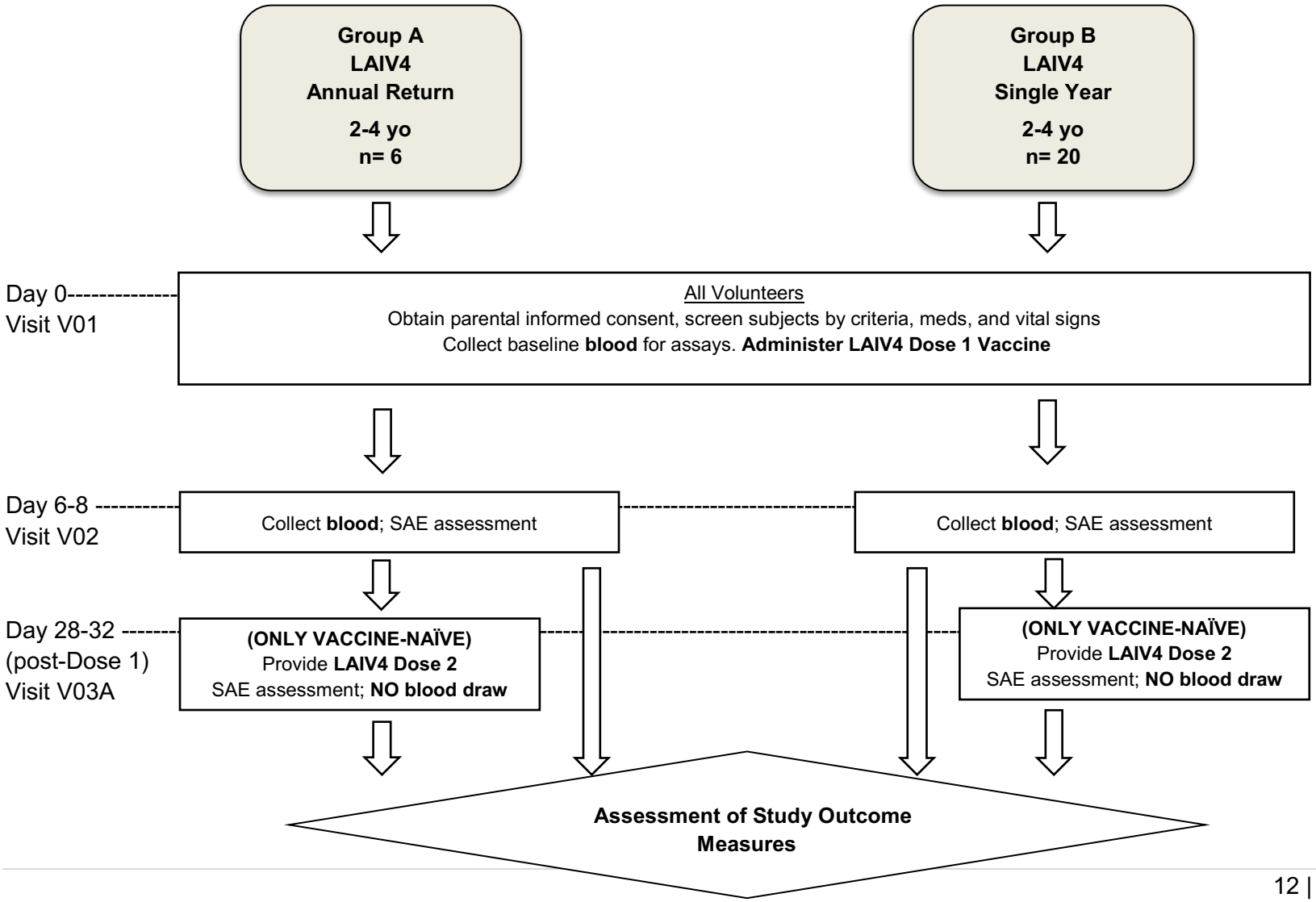
Group E (IIV4/single year): Up to twenty 6 month–23 month old (inclusive) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for children requiring 1 dose of vaccine (prior flu vaccination history). For children requiring 2 doses of vaccine (vaccine-naïve), a second immunization will be given at Day 28-32 after Dose 1. The second blood sample will be collected on Day 6-8 post- Dose 2. Volunteers will participate for single year.

Group F (LAIV4/single year) Up to forty 9-13 year old (n= 20) and 18-49 year old (n= 20) volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples and NP swabs will be

collected. Blood samples will be taken at Day 0 pre-immunization, Day 6-8 post-immunization and Day 28±4 post-immunization. NP swabs will be collected at Day 1 post-immunization and Day 28 post – immunization. Volunteers will participate for a single year. Group G (IIV4/single year) Up to forty 9-13 year old (n= 20) and 18-49 year old (n= 20) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct assays will be taken at Day 0 pre-immunization, Day 6-8 post immunization and Day 28±4 post-immunization. Volunteers will participate for single year. No NP swabs will be collected for this group.
Estimated Time to Complete Enrollment: Participants will be enrolled Aug-Dec in each of 5 years

U19 CCHI Project 2 (SLVP029)

Innate and Acquired Immunity to Influenza Infection and Immunization



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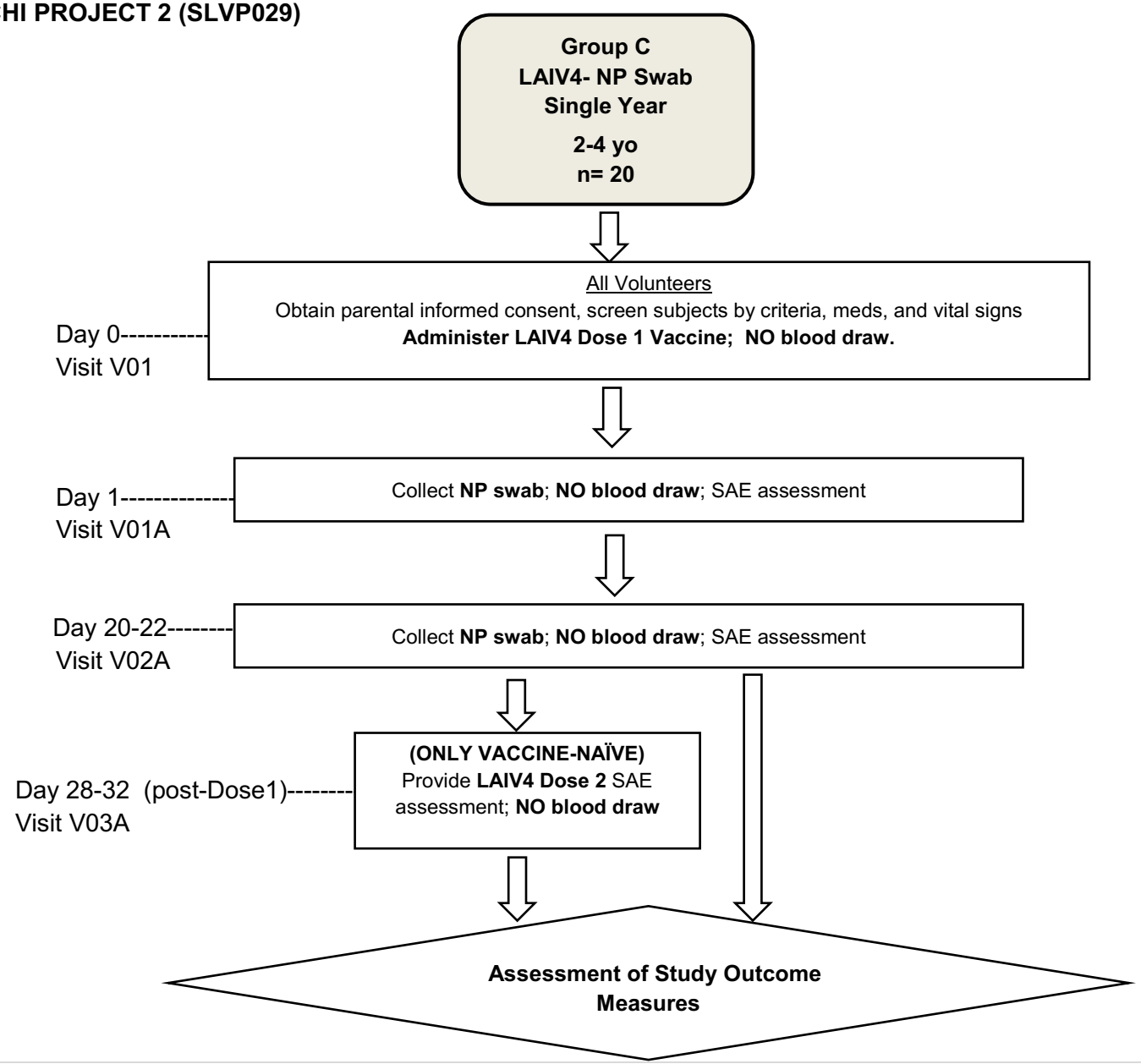

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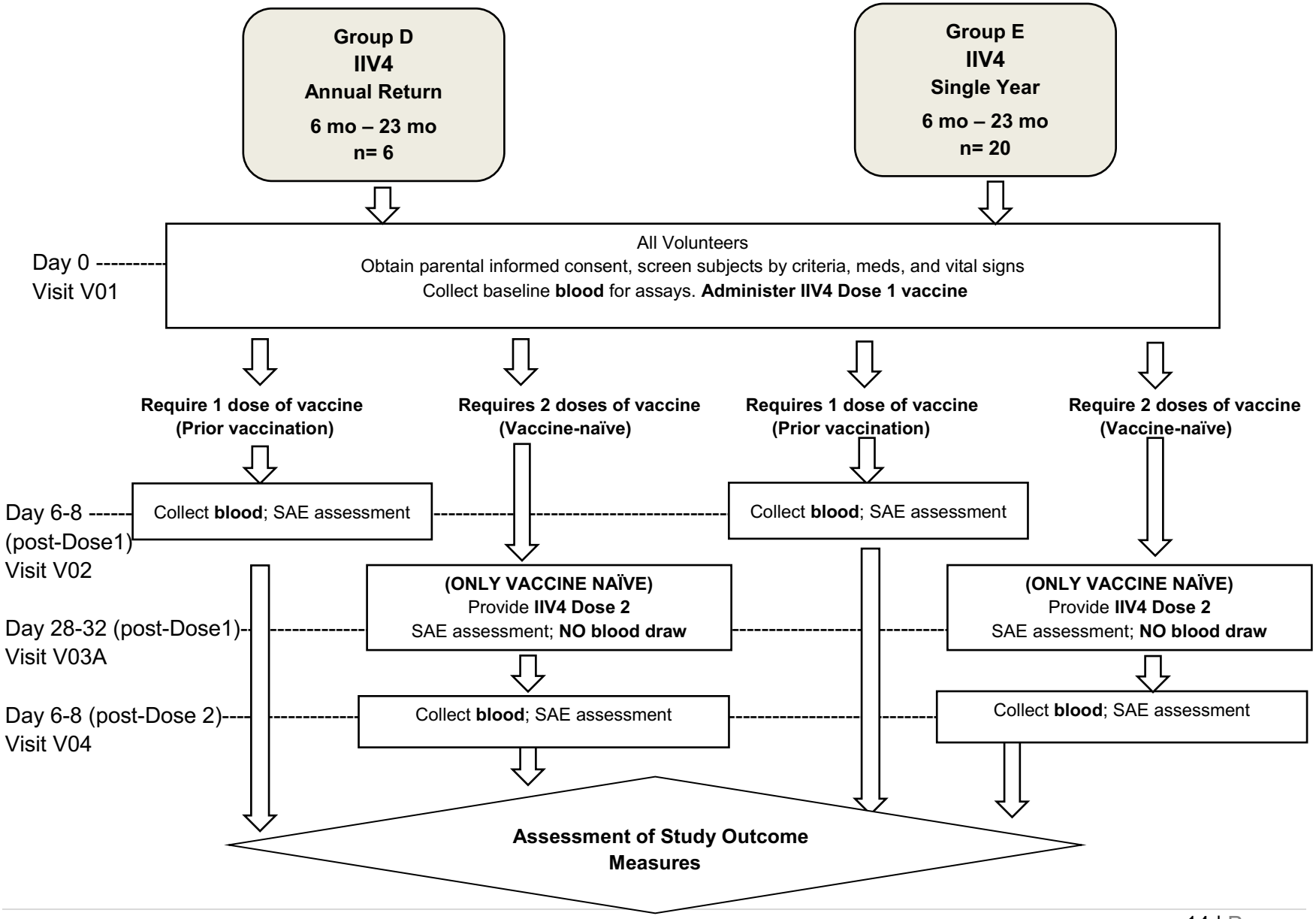

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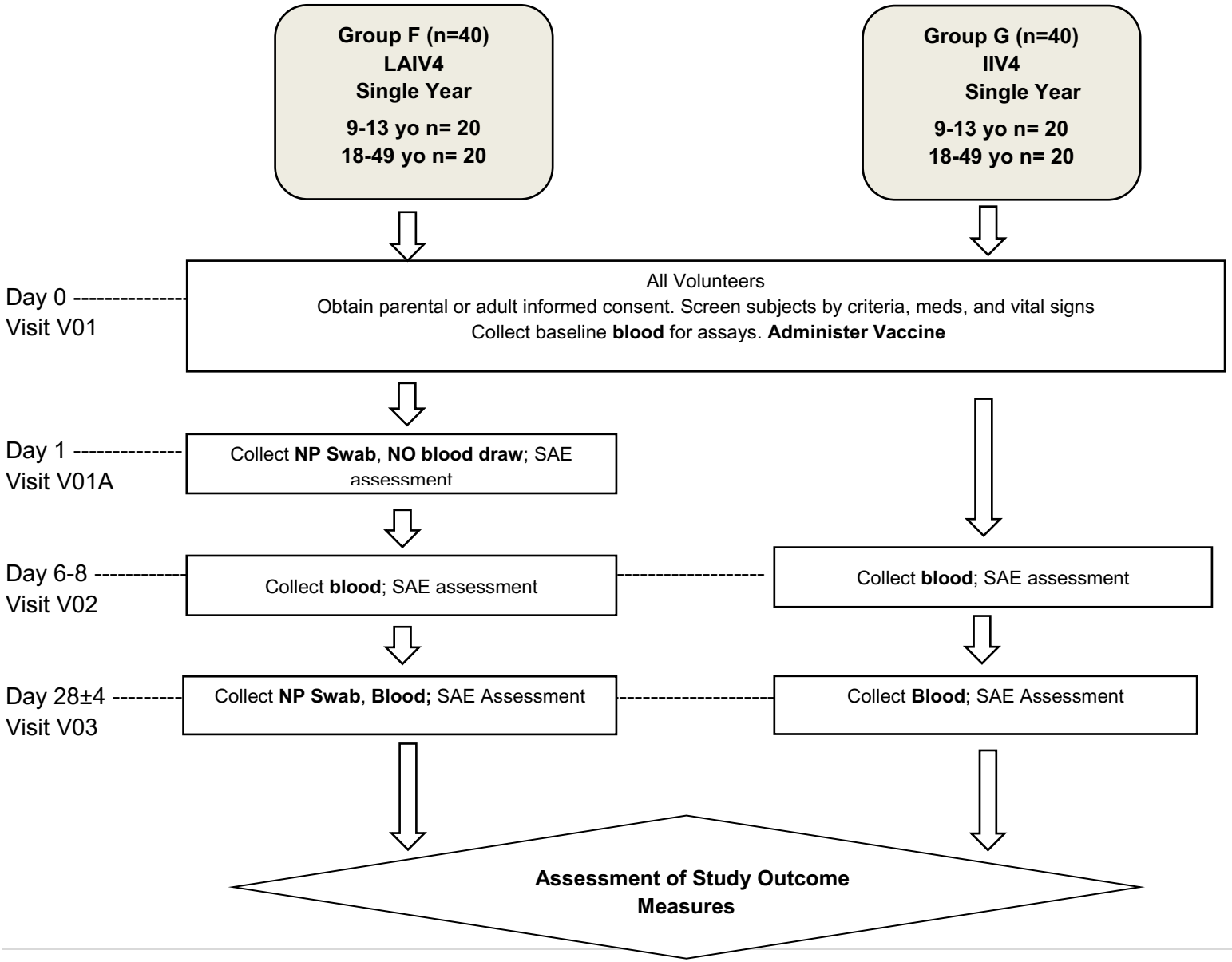

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Influenza virus (flu) is a Category C priority human pathogen with a worldwide distribution that is responsible for annual epidemics and intermittent pandemics. The spread of the 2009 swine-origin flu A/H1N1 pandemic and the identification of highly pathogenic avian A H5N1 and H7N9 strains highlight the need for better vaccines. Two types of licensed vaccines are available in the US: trivalent (IIV3, previously TIV) and quadrivalent (IIV4) inactivated vaccines both administered by injection, and nasally administered quadrivalent live, attenuated influenza vaccines (LAIV4) administered by nasal spray. Both vaccines are safe and effective in their approved age groups. LAIV is more efficacious than IIV, especially against mismatched flu strains, in children 6 months to 18 years of age[1-3], as well as in a recent study using ferret model[35]. In adults 18 to 49 years of age, LAIV has been either equally effective or somewhat less effective than IIV[1,4-5]. In the elderly, IIV has variable but often modest efficacy[6]. Despite extensive investigation of immune responses in infected patients and vaccine recipients, the basis of protective immunity is not fully understood. B cell and antibody (Ab) responses are key components of the adaptive immunity induced by natural (or "wild type", wt) flu infection and vaccination, but conventional measures of serum Ab do not always predict protective efficacy of flu vaccination, especially of LAIV in healthy adults and of IIV in the elderly. A recent study of experimentally infected adults showed that levels of flu-specific CD4 T cells correlated with reduced virus shedding and disease severity[7], suggesting that preexisting cellular immunity plays a role in modulating viral replication and pathogenesis. In previous studies we showed that LAIV induced greater T cell responses in children than in adults[8].

The upper respiratory tract (URT) mucosa is the initial replication site of wt flu and LAIV. The nasal mucosa is a complex and well-integrated system that serves as a first line of defense against infection[9]. The URT mucosa contains several types of epithelial cells thought to be the targets of initial infection by respiratory viruses including flu[10]. In a recent study, nasal swab specimens from flu patients were successfully used to identify lipid mediators that both induce and resolve local inflammation[11]. Previous studies have shown robust innate immune responses in primary human alveolar and bronchial epithelial cells infected by flu *ex vivo*[12-14]. Studies in mice and humans suggested that early innate immune responses help shape subsequent adaptive responses. This is supported by the finding that the magnitude and persistence of Ab responses, as well as subsequent recall responses, to a live attenuated yellow fever vaccine correlate with early innate immune transcriptional responses[15-16] and likely involve multiple toll-like receptors that stimulate production of proinflammatory cytokines[17-18].

Animal studies have shown that type I interferon (IFN) responses elicit rapid and significant amounts of antigen (Ag)-specific IgG2c predominantly from follicular B cells[19], and are important for the optimal priming, expansion, and memory formation of T cells after flu infection[20-21]. We recently found that rotavirus (RV) infection of intestinal enterocytes *in vivo* results in distinct transcriptional responses in infected vs. bystander uninfected epithelial cells and that most of the early IFN response to infection occurs in a nonepithelial immune cell compartment[22]. We also found that the local IgA B cell response to RV infection is promoted by plasmacytoid dendritic cells (DCs), a major producer of IFN during the early RV infection in both mice and humans[23]. These findings support the notion that early local innate immune responses, characterized by the robust induction of IFN and/or other cytokines, in the flu-infected nasal mucosa are likely to have a profound effect on viral replication and on subsequent virus-specific adaptive responses. This hypothesis will be tested in proposed experiments to examine the crosstalk between nasal epithelial and immune cell subsets, the effects of preexisting acquired immunity on this crosstalk, and the modulating effect the crosstalk has on viral replication and subsequent acquired immunity, presumably because children have lower levels of pre-vaccination immunity, resulting in higher levels of LAIV replication and subsequent T cell responses. The exact mechanisms linking preexisting immunity to local flu replication and subsequent immune responses are not clear.

The URT mucosa also hosts multiple resident professional Ag presenting cells and Ag-specific lymphocytes critical for adaptive and innate immune responses. These cells, including DCs, natural killer (NK) cells, and memory and effector B and T cells (including those specific for flu), can be collected using flocked nasopharyngeal swabs[24-25]. The outcome of LAIV immunization in flu-experienced older children and adults is likely strongly influenced by pre-existing flu-specific resident memory T and B cells in addition to the local innate responses. However, human URT resident immune cells have not been extensively studied and their role in controlling local flu replication as well as their relationship with local innate responses are unclear, largely due to difficulties in access and isolation of sufficient numbers of nasal cells for analysis. Defining the roles of these early adaptive immune cell responders to natural (or "wild-type", wt) flu and LAIV infection in the URT will be an important step toward identification of the modulators of local replication and immunity.

Ideally, a flu vaccine should induce protective immunity not only to homologous vaccine Ags but also to heterovariant strains or antigenically drifted new flu Ags. LAIV is clearly more effective at inducing protection against both antigenically matched and mismatched strains than IIV, especially in children[1,3]. However the basis for the enhanced induction of heterovariant protective immunity following infection (vs. parenteral vaccination) is not understood. It is not known whether heterovariant Ab reactivity is mediated by the same Ab molecules that recognize both homotypic and variant epitopes of different strains, or by different Ab molecules each targeting a cognate Ag on a specific variant strain. This question cannot be easily addressed with serum samples and conventional serologic assays. IIV induces a higher titer serum Ab

response than LAIV in adults and older children, but one year after vaccination with either LAIV or IIV the levels of flu-specific circulating memory B cells are similar[26]. This indicates that convalescent serum Ab titers do not give a complete picture of B cell mediated immunity following vaccination.[26-32] and infection[30, 33-34]. We and others have studied the B cell response to flu vaccination by examining the acute circulating plasmablast (PB) response. Analysis of this response provides a snapshot of the overall B cell response to recent Ag exposure without the confounding effects of preexisting circulating Abs. Analysis of the PB Ab repertoires revealed that the B cell response of adults to flu vaccination is largely a recall response of pre-existing memory B cells[28]. In preliminary studies, we identified flu-specific PBs that recognized previously circulated flu viruses better than current vaccine strains. These observations suggest that both the quantitative and qualitative nature of the B cell response to a new flu vaccination or to infection in the flu-experienced individuals is fundamentally shaped by the pre-vaccination memory B cell repertoire. However, factors that initially establish this repertoire and the evolution of this repertoire during repeated exposure to influenza antigens, especially during repeated immunization with different types of influenza vaccines, the mechanism of its regulation, and the impact of initial Ag exposure on subsequent flu immunity in different age groups are unknown.

2.2 Rationale

Our previous studies have focused on vaccine-induced immunity to influenza (flu); we have examined systemic humoral and cellular responses, as measured by levels of circulating and plasmablast-derived antibodies (Abs), T cells, B cells and natural killer cells, to identify determinants of immunity following intranasally-administered live attenuated (LAIV) and parenterally administered inactivated flu vaccines (IIV). The mechanistic basis for variable rates of protective immunity induced by vaccination – especially heterotypic immunity induced by LAIV in children and decreased immunity observed upon vaccination of the elderly with IIV – remains poorly understood. The upper respiratory tract (URT) is the initial site of replication of natural (or "wild type", wt) flu and LAIV strains. We hypothesize that key determinants of flu immunity are regulated at the URT level and can be understood by careful examination of virus-host interactions at the local level. These local determinants include early host antiviral innate responses, tissue-resident immune cells, and viral replication levels. We have initiated studies of early innate and acquired immune responses in primary human nasal cells collected either directly from patients acutely infected with influenza or subjects who had received LAIV, and in primary URT nasal cells acutely infected with wt flu or LAIV strains.

As a second approach to better understanding flu immunity, we have examined B cell and Ab responses to flu vaccination with a focus on qualitative and quantitative differences in responses between old and young vaccinees, IIV and LAIV recipients, and heterovariant vs. homotypic Ab reactivities. We propose to extend these studies using a

novel barcoding technology for high-throughput analysis of immunoglobulin (Ig) repertoires in flu-specific plasmablasts focusing on the initial responses to IIV vs. LAIV in influenza-naïve children. Evolutionary trees of Ab repertoires will be generated allowing us to identify – in an unbiased manner – affinity-matured clonal Ig families that are likely critical components of the flu Ab response. By expressing representative Ig genes from these clonal families, their functional property and homotypic vs. heterovariant specificity will be characterized.

Based on these two complementary research goals, we hypothesize the following: Infection of the URT with LAIV4 or wt virus triggers hierarchical transcriptional signatures in local resident epithelial and immune cells that regulate viral replication and subsequent immunity. We will collect NP swab specimens from recipients of LAIV4 and examine selected early transcriptional responses and viral replication levels in epithelial and lymphocytic cells (collection of the samples from subjects infected with the wild-type influenza virus will be completed by enrolling volunteers under a separate protocol). Host transcriptional responses and viral replication will be measured in bulk tissue, purified epithelial and lymphocyte cells, and single infected and bystander (non-infected) cells using flow cytometry and qRT-PCR. We predict that LAIV4 and wt virus will induce overlapping but clearly distinct transcriptional and phosphorylation signatures. In the older children and adult subjects from whom both NP swab and blood samples will be collected, we will also test whether local antiviral transcriptional responses and viral replication levels correlate with subsequent flu specific immune responses and will identify early local transcripts that predict subsequent B and T cell immunity in vivo.

We also hypothesize that: The antigen-specific Ig repertoires elicited by infection with wt flu virus or LAIV4 vs. immunization with inactivated flu vaccine (IIV4) are quantitatively and qualitatively distinct, especially in young children undergoing the first or second exposure to flu antigens, and are different from those in flu-experienced adults in terms of number, size, Ig gene usage, and isotype of clonally expanded Ig gene families, and in levels of somatic hypermutation and heterovariant cross-reactive Ab activity. We predict these differences have the potential to affect the evolution of an individual's immune response to novel flu antigens. In this protocol, we will examine these characteristics in LAIV4-infected vs. IIV4-exposed young children 6 months to 4 years of age who are influenza-naïve (or almost naïve) and compare them to children 9 – 13 years old and adults using a novel barcoding-based amplification and sequencing technology. These differences are predicted to shape the subsequent Ab and memory B cell responses, in particular their ability to recognize heterovariant strains.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

This protocol will immunize children and adult participants with either IIV4 (IM) or LAIV4 (intranasal) vaccines that are licensed 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 and collection of the mucosal tissue using a nasopharyngeal swab. 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. Persons who care for severely immunosuppressed persons who require a protective environment should not receive LAIV, or should avoid contact with such persons for 7 days after receipt, given the theoretical risk for transmission of the live attenuated vaccine virus to close contacts. It is not known whether LAIV4 (FluMist Quadrivalent) is excreted in human milk. Because some viruses are excreted in human milk, a lactating woman is not eligible to be enrolled in Group F.

2.3.2 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.

3 OBJECTIVES

3.1 Study Objectives

Primary:

1. To measure the nasal mucosal transcriptional responses to LAIV4 in the following age groups: 2-4 year old children, 9-13 year old children, and 18-49 year old adults.
2. To measure the B cell response to LAIV4 in the following age groups: 2-4 year old children, 9-13 year old children, and 18-49 year old adults.
3. To measure the B cell response to IIV4 in the following age groups: 6 month-4 year old children, 9-13 year old children, and 18-49 year old adults.

Secondary:

1. To compare the nasal mucosal transcriptional responses to LAIV4 between the different age groups.
2. To determine the evolution of B cell repertoire in young children after repeated immunization with different types of influenza vaccines.
3. To compare the B cell repertoire in response to LAIV4 versus IIV4 in different age groups.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures:

- mRNA levels,
- Ig gene sequence

3.2.2 Secondary Outcome Measures:

- HAI titers
- PPAb titer
- mAb reactivity

4 STUDY DESIGN

This is a Phase IV study of healthy children and adults who will receive the current seasonal influenza vaccine. There are no exclusions for gender, ethnicity or race. The volunteers will be enrolled into one of 7 groups over a 5 year period. Following confirmation of written informed consent and assent, immunization will be administered; blood samples and NP swabs for immunogenicity assays will be collected at various time points based on groups assigned. All volunteers will be followed for serious adverse events (SAEs).

Group A (LAIV4/annual return): Up to six 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for all children irrespective of those who require 1 dose (prior flu vaccination history) or 2 doses (vaccine-naïve). For children requiring 2 doses of vaccine, a second immunization will be given at Day 28-32 after Dose 1. All participants in this group will be asked to return annually for flu immunization and blood samples.

Group B (LAIV4/ single year): Up to twenty 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for all children irrespective of those who require 1 dose (prior flu vaccination history) or 2 doses (vaccine-naïve). For children requiring 2 doses of vaccine, a second immunization will be given at Day 28-32 after Dose 1.

Group C (LAIV4/NP swab group): Up to twenty 2-4 year old volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). NP swabs will be collected at Day 1 and Day 21 post-Dose 1 for all children irrespective of those who require 1 dose or 2 doses of vaccine. For children requiring 2 doses of vaccine, a second immunization will be given at Day 28-32 after Dose 1. No blood will be collected for this group.

Group D (IIV4/annual return): Up to six 6 month-23 month old (inclusive) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for children requiring 1 dose of vaccine (prior history of flu immunization). For children requiring 2 doses of vaccine (vaccine-naïve), a second immunization will be given at Day 28-32 after Dose 1. The second blood sample will be collected on Day 6-8 post-Dose 2. Volunteers will be asked to return annually for flu immunization and blood samples.

Group E (IIV4/single year): Up to twenty 6 month–23 month old (inclusive) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct the assays described will be taken at Day 0 pre-immunization and Day 6-8 post-Dose 1 for children requiring 1 dose of vaccine (prior history of flu immunization). For children

requiring 2 doses of vaccine (vaccine-naïve), a second immunization will be given at Day 28-32 after Dose 1. The second blood sample will be collected on Day 6-8 post- Dose 2. Volunteers will participate for single year.

Group F (LAIV4/single year) Up to forty 9-13 year old (n= 20) and 18-49 year old (n= 20) volunteers will be given a quadrivalent live, attenuated influenza vaccine (LAIV4). Blood samples and NP swabs will be collected. Blood samples will be taken at Day 0 pre-immunization, Day 6-8 post-immunization and Day 28±4 post-immunization. NP swabs will be collected at Day 1 post-immunization and Day 28 post –immunization. Children 9 yrs and older, and adults do not require a second dose of flu vaccine if previously unimmunized. Volunteers will participate for single year.

Group G (IIV4/single year) Up to forty, 9-13 year old (n= 20) and 18-49 year old (n= 20) volunteers will be given a quadrivalent inactivated influenza vaccine (IIV4). Blood samples to conduct assays will be taken at Day 0 pre-immunization, Day 6-8 post immunization and Day 28±4 post-immunization. No NP swabs will be collected for this group. Children 9 yrs and older, and adults do not require a second dose of flu vaccine if previously unimmunized. Volunteers will participate for single year.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

1. Otherwise healthy, 6 mo-49 years old volunteers
2. Willing to complete the informed consent process (including assent for minors 7-17 years old).
3. Availability for follow-up for the planned duration of the study.
4. For parents of children 6 months – 4 years of age: Willing to participate in the study annually for up to 5 years (if yes, consider for annual return groups).
5. Acceptable medical history by review of inclusion/exclusion criteria and vital signs.
6. Influenza vaccine-naïve or only one prior season of flu immunization with IIV (does not apply to Groups F and G).

5.2 Subject Exclusion Criteria for Enrollment and Subsequent Annual Vaccinations

1. Prior off-study vaccination with the current year's seasonal influenza vaccine
2. Receipt of LAIV in the prior season (does not apply to Groups F and G)
3. Received flu immunizations in 2 or more prior flu seasons (does not apply to Groups F and G)
4. Allergy to egg or egg products, or to vaccine components, including gentamicin, gelatin, arginine or MSG (if will be given LAIV4)
5. Life-threatening reactions to previous influenza vaccinations
6. Asthma in adults. Children aged 2 through 4 years who have asthma or who have had a wheezing episode noted in the medical record within the past 12 months, or for whom parents report that a health care provider stated that they had wheezing or asthma within the last 12 months [If yes, not eligible for LAIV Groups A, B, C, & F].
7. Active systemic or serious concurrent illness, including febrile illness on the day of vaccination
8. History of immunodeficiency (including HIV infection)
9. For children or adolescents through 17 years of age: receiving aspirin therapy or aspirin-containing products [If yes, not eligible for LAIV Groups A, B, C, and F].
10. Known or suspected impairment of immunologic function, including, but not limited to, clinically significant liver disease, diabetes mellitus treated with insulin, moderate to severe renal disease, or any other chronic disorder which, in the opinion of the investigator, might jeopardize volunteer safety or compliance with the protocol.
11. Blood pressure >150 systolic or >95 diastolic at first study visit and the day of vaccination (for children 12 yrs and older, and adults).
12. Hospitalization in the past year for congestive heart failure or emphysema.
13. Chronic Hepatitis B or C
14. Recent or current use of immunosuppressive medication, including systemic glucocorticoids (corticosteroid nasal sprays and topical steroids are permissible in all groups; inhaled steroid use is not permissible)
15. Persons who care for severely immunosuppressed persons who require a protective environment should not receive LAIV, or should avoid contact with such persons for

7 days after receipt, given the theoretical risk for transmission of the live attenuated vaccine virus to close contacts. [If yes, may be ineligible for Groups A,B, C and F].
16. Malignancy, other than squamous cell or basal cell skin cancer (includes solid tumors such as breast cancer or prostate cancer with recurrence in the past year, and any hematologic cancer such as leukemia).
17. Autoimmune disease (including rheumatoid arthritis treated with immunosuppressive medication such as Plaquenil, methotrexate, prednisone, Enbrel) which, in the opinion of the investigator, might jeopardize volunteer safety or compliance with the protocol
18. History of blood dyscrasias, renal disease, or hemoglobinopathies requiring regular medical follow up or hospitalization during the preceding year
19. Use of any anti-coagulation medication such as Coumadin or Lovenox, or anti-platelet agents such as aspirin (except up to 325 mg. per day), Plavix, or Aggrenox must be reviewed by investigator to determine if this would affect the volunteer's safety.
20. Has taken an influenza antiviral medication within 48 hours prior to study vaccination [If yes, may not eligible for LAIV Groups A, B, C and F if unable to reschedule study vaccination].
21. Receipt of blood or blood products within the past 6 months or planned used during the study
22. Medical or psychiatric condition or occupational responsibilities that preclude participant compliance with the protocol
23. Receipt of inactivated vaccine 14 days prior to study enrollment, or planned vaccinations prior to completion of last study visit
24. Receipt of live, attenuated vaccine within 60 days prior to enrollment of planned vaccination prior to completion of last study visit
25. Need for allergy immunization (that cannot be postponed) during the study period
26. History of Guillain–Barré syndrome
27. Pregnant woman
28. Breastfeeding [If yes, not eligible for LAIV Group F]
29. Use of investigational agents within 30 days prior to enrollment or planned use during the study
30. Donation of the equivalent of a unit of blood within 6 weeks prior to enrollment or planned donation prior to completion of the last visit
31. 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

Volunteers will not be randomized to study treatment. All eligible volunteers will receive either quadrivalent live, attenuated influenza vaccine (LAIV4) or quadrivalent inactivated influenza vaccine (IIV4) based upon eligibility and group assigned.

5.3.2 Reasons for Withdrawal

A study participant will not receive the study product at the first visit if a significant intercurrent illness, or other medical condition or situation occurs that meets the exclusion criteria. Volunteers will be withdrawn from the study if participation in the study would not be in the best interest of the participant, or if doing so would harm the participant in the opinion of the investigator. Participants may withdraw from the study voluntarily.

5.3.3 Handling of Withdrawals

If, for safety reasons a participant is deemed by the investigators to not be eligible to receive the study product as per protocol, he/she will be terminated from the study. If a participant voluntarily withdraws from the study or is withdrawn by the investigator after immunization, the participant will be followed for safety whenever possible until Day 28 post-immunization or until any serious adverse events are resolved or considered stable.

5.3.4 Termination of Study

The study may be terminated for administrative reasons or other unanticipated circumstances.

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. The quadrivalent IIV4 will be supplied by Sanofi-Pasteur as Fluzone® Quadrivalent. The LAIV4 will be supplied by MedImmune as FluMist® Intranasal spray, Quadrivalent Influenza Vaccine.

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. Each 0.25 mL dose contains a total of 30 µg (7.5 µg of each strain). The vaccine will be supplied in a prefilled, single dose syringe 0.25 mL (no preservative) 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 contains 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.

6.1.3 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). Should not use 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). One should not use after the expiration date shown on the label.

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

Fluzone® Quadrivalent: 0.25 mL for children 6 months to 35 months of age and 0.5 mL for 36 months – adults. IIV4 vaccine will be administered with a sterile, disposable syringe and needle by IM injection into the thigh or deltoid muscle. The participant/clinician will choose whether the injection will be administered into the thigh or right or left deltoid. Previously unvaccinated children through 8 years of age should receive two doses of influenza vaccine - one on day 0, followed by a second dose at least 28 days later.

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. Previously unvaccinated children through 8 years of age should receive two doses of influenza vaccine - one on day 0, followed by a second dose at least 28 days later.

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

The study article will be shipped by the manufacturer to the investigational pharmacy at 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 returned to the investigational pharmacy for use or destruction.

6.4 Concomitant Medications

At each study visit, the participant will be questioned about any vaccinations they have received or concomitant medication use, and the information will be recorded. Medication history will include medications taken within 28 days prior to enrollment and currently taken 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), anti-coagulation/anti-platelet medications such as Coumadin, Lovenox, aspirin (except aspirin up to 325 mg. per day), Plavix, Aggrenox, or any other medications which might indicate a condition that precludes participant compliance with the protocol.

7 STUDY SCHEDULE

7.1 Screening

Volunteers will be contacted by phone prior to the first study visit. Study staff will briefly review the eligibility criteria, participant availability and the study procedures. If the participant is eligible and interested, the volunteers will be assigned to groups based on, age, inclusion exclusion criteria and availability for annual return vs single year participation.

7.2 Day 0; Enrollment/Baseline/Immunization for all Groups; annually for Groups A and D.

- Adult participants or parents of the volunteer will be provided with a verbal description of the study (purpose and study schedule and procedures). They then will be asked for any questions and to read/sign the consent form (in the first year only). Minor children ages 7-17 years will be given an assent at enrollment. The consent form will be signed prior to the performance of any study procedures. The study staff will discuss with the volunteer or volunteer's parent of their study eligibility criteria and concomitant medication use.

- Obtain vital signs (oral temperature, pulse, and respiratory rate), height and weight. Obtain blood pressure for volunteers 12 years and older.

- Collect blood samples **(except Group C)** as follows:

  For blood volumes $\geq$ 20 mL, collect one 5 mL gold top tube (serum), one 2.5 mL PAXgene tube and one 2.5 mL heparinized, green top tube (CyTOF sample). The remainder of the blood sample will be collected in heparinized, green top tubes. Blood volumes <20 mL will be collected in heparinized, green top tubes only.

  - For children weighing more than 100 lb., and from adults, total volume not to exceed 70 mL
  - From children weighing 66-100 lb: total volume not to exceed 30 mL
  - From children weighing 61-65 lb: total volume not to exceed 25 mL
  - From children weighing 41-60 lbs: total volume not to exceed 20 mL
  - From children weighing 16-40 lbs: total volume not to exceed 10 mL
  - From children 6 months through 35 months old, regardless of weight: Obtain a 4 mL blood sample into a heparin (green top) tube.

- All volunteers will be administered the seasonal flu vaccine according to the group assignment. Unless there is a different recommendation from the ACIP in a given year, Group A, B, C and F will receive intranasal spray of LAIV4. Group D, E and G

will be vaccinated with IIV4. Children who are vaccine-naïve will receive their Dose 1 of their vaccine at this visit. Volunteers will be observed in clinic for 15 minutes for any immediate serious reactions. Vaccine-naïve volunteers requiring Dose 2 will return at least 28 days post-Dose 1.

- A Memory Aid will be given to the volunteers/volunteer's parent with written and oral instructions for collection of information about serious adverse events. The memory aid is a simple diary for volunteer to record change in a health status and medications between study visits.

- The schedule for subsequent study visits will be reviewed.

7.3 Day 1 post- Dose 1 immunization for Group C and Group F only

- Review of medications and memory aid for serious adverse events

- Collect nasopharyngeal swab specimen; no blood sample will be collected at this visit.

- The schedule for subsequent study visits will be reviewed.

7.4 Day 6-8 post-Dose 1 immunization <u>except</u> Group C and vaccine-naïve volunteers in Group D and Group E requiring Dose 2; annually for Groups A and D.

- Review of medications and memory aid for serious adverse events

- Collect blood samples as follows:

  All blood samples will be collected in heparinized, green top tubes to be picked up by the Greenberg lab for processing and analysis. For sample volumes >30 mL, one heparinized, green top tube will be sent to the HIMC if available (Greenberg lab requires at least 30 mL in heparinized, green top tubes at Day 6-8).

  - For children weighing more than 100 lb., and from adults: total volume not to exceed 70 mL
  - From children weighing 66-100 lb: total volume not to exceed 30 mL
  - From children weighing 61-65 lb: total volume not to exceed 25 mL
  - From children weighing 41-60 lbs: total volume not to exceed 20 mL
  - From children weighing 16-40 lbs: total volume not to exceed 10 mL
  - From children 6 months through 35 months old, regardless of weight: Obtain a 4 mL blood sample into a heparin (green top) tube.

- The schedule for subsequent study visits will be reviewed for, Group F and G

- This will be the final visit for volunteers with prior influenza vaccination history in Group A, B, D and E. Complete Study Status form and Study Reimbursement form for these volunteers.

7.5 Day 21 post-Dose 1 immunization for Group C only

- Review of medications and memory aid for serious adverse events
- Collect nasopharyngeal swab specimen.
- No blood sample will be collected at this visit.
- This will be the final visit for volunteers with prior influenza vaccination history. Complete Study Status form and Study Reimbursement form for these volunteers.

7.6 Day 28 post-Dose 1 immunization

7.6.1 Day 28-32 for vaccine-naïve volunteers requiring second dose of vaccine (Groups A, B, C, D and E)

- Review of medications and memory aid for serious adverse events
- Obtain temperature.
- No blood samples will be collected at this visit
- Volunteers will be vaccinated with the same type of vaccine as Dose 1 and observed in clinic for at least 15 minutes for any immediate serious adverse events.
- Complete Study Status form and Study Reimbursement form for Groups A, B and C.
- For Groups D and E Memory aid will be given with written and oral instructions for collection of information about serious adverse events.
- The schedule for subsequent study visit will be reviewed for Group D and E

7.6.2 Day 28 ± 4 for Groups F and G

- Review of medications and memory aid for serious adverse events
- Collect nasopharyngeal swab specimen for Group F only
- Collect blood samples for both group as follows:

  For blood volumes $\geq$ 20 mL, collect one 5 mL gold top tube (serum). The remainder of the blood sample will be collected in heparinized, green top tubes. Blood volumes

<20 mL will be collected in heparinized, green top tubes only.

- For children weighing more than 100 lb., and from adults, Volume not to exceed 70 mL
- From children weighing 66-100 lb., total volume not to exceed 30 mL
- From children weighing 61-65 lb., total volume not to exceed 25 mL
- From children 6 months through 35 months old, regardless of weight: Obtain a 4 mL blood sample into a heparin (green top) tube.

- Complete Study Status form and Study Reimbursement form

7.7 Day 6-8 post-Dose 2 immunization for previously vaccine-naïve volunteers in Group D and E

- Review of medications and memory aid for serious adverse events
- Collect blood samples for both group as follows:

  All blood samples will be collected in heparinized, green top tubes to be picked up by the Greenberg lab for processing.

  - From children weighing 41-60 lbs. total volume not to exceed 20 mL
  - From children weighing 16-40 lbs. total volume not to exceed 10 mL
  - From children 6 months through 35 months old, regardless of weight: Obtain a 4 mL blood sample into a heparin (green top) tube.

- Complete Study Status form and Study Reimbursement form

7.8 Early Termination Visit

If a volunteer is terminated from the study early, every effort should be made to perform the following procedures:

- Review current health status and note any changes since the last visit. Solicit information regarding SAEs and record all concomitant medications. Any ongoing related SAEs will be followed to resolution or until a stable chronic condition has been established. Volunteers will be encouraged to permit continued follow-up of SAEs if necessary
- Obtain remaining samples, if possible.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Clinical Evaluation will be obtained by interview with a research nurse, and will include:

- Review eligibility and past medical history including influenza vaccine history.
- Medication history of medications used within 28 days prior to enrollment and currently taken prescription and over-the-counter medications used throughout the study period.
- Height, weight, temperature, pulse, BP on adolescents and adults.
- Review of memory aid for serious adverse events occurring during the 28 day study period.

8.2 Laboratory Evaluations

8.2.1 Special Assays or Procedures

Subject Analysis – We will use CyTOF mass cytometry to determine the precise number of each white blood cell type in a given patient sample and their activation state and function (B cells, T cells, NK cells, T cells, monocytes, dendritic cells).

Gene Expression Analysis– We will collect part of the blood sample in a PAXgene tube and prepare probes from the mRNA for gene expression analysis using Agilent microarrays that survey global gene expression. This will tell us if there is a particular gene expression signature.

Serum Cytokine Analysis – As there have been reports of changes in the serum cytokine repertoire in older people, we will survey serum samples for different cytokines using the Panoics/Luminex system now running the Human Immune Monitoring Center at Stanford.

Responses to Cytokine Stimulation – We will stimulate PBMCs with various cytokines and will analyze the phosphorylation status of different STATs and other key mediators of cellular activation in B cells, T cells and monocytes by flow cytometry.

HAI Antibody Titer – The standard HAI antibody assay will be run on samples at baseline and Day 28 or other study visit to evaluate the duration of response to vaccination.

Sequencing of TCR$\alpha\beta$ and Immunoglobulin Genes– We will sort B and T cells and sequence their antibody repertoires.

Host transcriptional response - We will carry out quantitative real time PCR analysis to determine the levels of specific transcripts in bulk and sorted nasal cell populations.

Antibody reactivity – We will generate plasmablast-derived polyclonal antibodies (PPAb) and recombinant mAbs and determine their reactivity against influenza antigens of the vaccine and variant strains by ELISA and SPR assays.

8.2.2 Specimen Preparation, Handling, and Shipping

Serum

Red top or Gold Top serum separator tube:
Procedural recommendations:

- Slowly invert tubes 8-10 times immediately after collection
- Store upright at room temperature until centrifugation
- Centrifuge within 2 hrs. of collection at 1100 RCF for 15 minutes at room temperature
- Aliquot and freeze at -20C within 2 hrs. of collection, and transfer to -80C within 2 weeks .
- An aliquot of serum may be sent to Hana Golding at the FDA for analysis in the future.

PBMCs

Collect whole blood into heparinized green top tubes for flow cytometric analysis of PBMC subsets and phospho-proteins, stimulation assays (cytokine production and gene expression), MHC tetramer arrays.

Tilt tubes immediately after filling and deliver to CTRU or HIMC lab for processing

RNA

2.5 ml in a PAXgene Blood RNA Tube for transcriptional (mRNA) profiling using microarrays, MicroRNA analysis, quantitative PCR (qPCR) analysis of mRNA expressed in peripheral blood.
Recommendations:

- Should be drawn as 2nd or later tube (not first)
- Invert 3 – 5 times immediately following collection
- Freeze within 1 hour at -20°C or -70°C, long term storage at -70°C prior to processing
- Do not freeze PAXgene Blood RNA Tubes standing upright; instead place the tubes horizontally in a plastic bag or tray for freezing.

NP swabs

NP swab specimens will be collected using standard flocked swabs, following the manufacturer's instruction.

8.3 Specification of Safety Parameters

Serious adverse events will be assessed through the last visit and followed to resolution or stability.

All SAEs occurring while the volunteer is on study will be collected and appropriately reported.

8.4 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

8.4.1 Serious Adverse Events

Serious Adverse Event (SAE): An SAE is defined as an adverse event (AE) that meets one of the following conditions:

- Death during the period of protocol defined surveillance
- Life-threatening event (defined as a participant at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- Reviewed and evaluated by a study clinician
- Recorded on the appropriate AE reporting form
- Reported to the Stanford IRB as required
- Followed through resolution by a study clinician

8.5 Reporting Procedures

8.5.1 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.

8.5.2 Type and Duration of Follow-up of Subjects after Adverse Events

All SAEs will be followed until satisfactory resolution or until the PI or Sub-investigator deems the event to be chronic or the patient to be stable.

8.6 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 CLINICAL MONITORING

This study will not be monitored by the sponsor. This is an exploratory study using licensed products. The investigator is responsible to ensure compliance with the protocol, and the accuracy, completeness, legibility, and timeliness of the data reported.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

1. Infection of the URT with LAIV4 or wt virus triggers hierarchical transcriptional signatures in local resident epithelial and immune cells that regulate viral replication and subsequent immunity.

2. The antigen-specific Ig repertoires elicited by IIV4 are quantitatively and qualitatively distinct, especially in young children undergoing the first or second exposure to flu antigens, and are different from those in flu-experienced adults in terms of number, size, Ig gene usage, and isotype of clonally expanded Ig gene families, and in levels of somatic hypermutation and heterovariant cross-reactive Ab activity.

This is an exploratory study aimed at providing initial estimates for subsequent studies design aimed at identifying age-specific characteristics in B cell response to influenza vaccine.

10.2 Sample Size Considerations

This is an exploratory study using a strategy that has not been employed previously to investigate the nasal mucosal transcriptional responses and the Ig repertoire of B cell response to influenza vaccination. We plan to enroll a total of 20 volunteers in each age group. This projected sample size is comparable or larger to previous studies on Ig repertoire in human or zebra fish, which in general used relatively small numbers of participants to generate large numbers (10 – 50) of clones from each individual.

10.3 Final Analysis Plan

In this proposal, the host innate immune responses to influenza virus infection will be examined within flow cytometry defined subsets of primary infected and bystander cells, as well as in bulk nasal mucosa RNA preparations. We have recently developed statistical models and methods that are appropriate to analyze transcriptional profiles following viral infection in high dimensional datasets. The basic approach that we propose is to obtain bulk measures at different hierarchical levels of the human nasal mucosa, including from sorted bulk populations and single cells from selected populations, and to identify significant innate response transcriptional signatures in the different age groups. Detailed procedures and analysis routines for analysis have been published recently and will be followed[36]

We will obtain sequences of cognate heavy- and light-chain pairs and determine their V(D)J usage by analyzing them with IMGT HighV-QUEST. The heavy and light chains will be grouped according to their V-gene usage to clonal families of antibodies that use the same heavy-chain V(D)J and light-chain VJ sequences. Antibody phylogenetic trees will be generated based on the Ig sequences. The number, size, Ig gene usage, and isotype of clonally expanded Ig gene families, and in levels of somatic hypermutation and heterovariant cross-reactive Ab activity will be compared between the different vaccines and age groups.

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

12 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.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

The investigator 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).

13.2 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.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the participants and their families. Consent forms describing in detail the study procedures and risks are given to the participant and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB-approved and the participant or parent will be asked to read and review the document. After reviewing the document, the investigator or authorized study staff will explain the research study to the participant or parent and answer any questions that may arise.

The participant or parent will sign the informed consent document prior to being enrolled in the study. The participant or parent should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant or parent may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participant or parent for their 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.

13.3.1 Informed Consent/Assent Process (in Case of a Minor)

An assent form approved by the IRB will be discussed with minors and signed by minor participants who are 7 years of age or older (up to age 18 years) after the parent provides consent.

13.4 Exclusion of Women, Minorities, and Children (Special Populations)

There will be children 6 months – 13 years of age who will be recruited for this study as well as adults of differing ages. There will be no exclusions based on gender, race or ethnicity.

13.5 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.

13.6 Study Discontinuation

The investigators have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of serious adverse events indicating a potential health hazard
- Data recording is inaccurate or incomplete
- Study staff does not adhere to the protocol or applicable regulatory guidelines in conducting the study.

A participant may withdraw or may be withdrawn from the study for the following reasons:

- Participant withdraws consent
- Development of serious adverse event warranting withdrawal

- Trial termination
- Any reason that, in the opinion of the investigator, precludes the participant's participation in the study.

13.7 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.

14 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.

14.1 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.

14.2 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.

14.3 Types of Data

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

14.4 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.

14.5 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.

14.6 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.

15 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.

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SUPPLEMENTS/APPENDICES

Appendix A. Schedule of visits

Appendix B. Schedule of events

Appendix C. Season-specific FluMist Quadrivalent package inserts 2014-2018

Appendix D. Season-specific Fluzone Quadrivalent package insert 2014-2018

APPENDIX A: SCHEDULE OF VISITS

Groups	Naïve /prior vacc	Day 0	Day 1 Post-Dose 1	Day 6-8 Post-Dose1	Day 21 Post-Dose1	Day 28-32 post-Dose 1 (for Dose 2) Day 28 ± 4 Post-Dose 1 (blood draw, NP Swab)	Day 6-8 Post-Dose 2
Group A 2-4 yo Annuals	naive	LAIV4 Dose 1/ Blood	--	Blood	--	LAIV4 Dose 2	--
	Prior vacc	LAIV4/Blood	--	Blood	--	--	--
Group B 2-4 yo Single yr	Naive	LAIV4 Dose 1/ Blood,	--	Blood	--	LAIV4 Dose 2	--
	Prior vacc	LAIV4/Blood	--	Blood	--	--	--
Group C 2-4 yo Single yr	Naive	LAIV4 Dose 1	NP Swab	--	NP Swab	LAIV4 Dose 2	--
	Prior vacc	LAIV4	NP Swab	--	NP Swab	--	--
Group D 6-23mo Annual	Naive	IIV4* Dose 1/ Blood	--	--	--	IIV4* Dose 2	Blood
	Prior vacc	IIV4*/ Blood	--	Blood	--	--	--
Group E 6-23mo Single yr	Naive	IIV4 Dose 1/ Blood	--	--	--	IIV4 Dose 2	Blood
	Prior vacc	IIV4/ Blood	--	Blood	--	--	--
Group F 9-13 yo 18-49 yo	Prior vacc	LAIV4, blood	NP Swab	Blood	--	Blood, NP Swab	--
Group G 9-13 yo 18-49 yo	Prior vacc	IIV4, blood	--	Blood	--	Blood	--

* When these children turn 2 yrs old in annual f/u, they will be switched to receive LAIV4 vaccine unless they develop a contraindication.

APPENDIX B: SCHEDULE OF EVENTS

	Day 0	Day 1	Day 6-8 post-Dose 1	Day 21 post-Dose 1	Day 28-32 post-Dose 1 (give Dose 2) OR Day 24 - 32 Post-Dose 1 (blood draw)	Day 6-8 post-Dose 2
Obtain Informed Consent/Assent	X					
Eligibility	X					
Concomitant meds	X		X		X	
Vital Signs[1]	X		X[2]		X[2]	
Blood Draw	X		X		X[7]	X[6]
NP Swab		X[3, 4]		X[3]	X[4]	
Immunization	X				X[5]	
Clinical Assessment	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Review of SAEs	X	X	X	X	X	X

[1] Vital Signs: temperature, pulse, respiration; height and weight (blood pressure for adolescents as indicated and adults)
[2] As indicated
[3] Group C only
[4] Group F only
[5] Dose 2 vaccine (at least 28 days post-Dose 1), Vaccine-naïve volunteers in Groups A-E only
[6] Groups D and E vaccine-naïve volunteers only
[7] Groups F and G volunteers only

APPENDIX C: FLUMIST QUADRIVALENT PACKAGE INSERTS 2014-2018

Attached in eProtocol section 16

APPENDIX D: FLUZONE QUADRIVALENT PACKAGE INSERTS 2014-2018

Attached in eProtocol section 16