A comparison of CD4 T cell induction and antibody responses between a pure hemagglutinin influenza vaccine (rHA, Protein Sciences Corp) and licensed subvirion influenza vaccine made in eggs (Sanofi) or cell culture (Novartis) in healthy adults.

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Site Principal Investigator:

Angela Branche, MD

University of Rochester Medical Center

DMID Clinical Project Manager:

Melinda Tibbals

Version: 9.0

08 February 2021

Statement of Compliance

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

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- 21 CFR 312
- ICH GCP E6
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award

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.

Principal Investigator:

Signed: _____ Date: _____

Angela Branche, MD

Principal Investigator, University of Rochester

CEIRS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
FWA	Federalwide Assurance
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
JAMA	Journal of the American Medical Association
MOP	Manual of Procedures
N	Number (typically refers to participants)
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
WHO	World Health Organization

1 PROTOCOL SUMMARY

Title: A comparison of CD4 T cell induction and antibody responses between a pure hemagglutinin influenza vaccine (rHA, Protein Sciences Corp) and licensed subvirion influenza vaccine made in eggs (Sanofi) or cell culture (Novartis) in healthy adults.

Population: Healthy adults ages 18 to 49 years

Number of Sites: Single site study

Study Duration: 7 years

Subject Duration: 6 months

Agents: Flublok (baculovirus expressed HA), Flucelvax (inactivated vaccine generated in mammalian cell culture, Fluzone SD (inactivated vaccine generated in eggs, administered at 15 ug per HA), Fluzone HD (inactivated vaccine generated in eggs, administered at 60 ug per HA)

Primary Objective

- Comparison of the serum antibody response to immunization with pure HA, cell- derived and egg derived subvirion vaccines in adults age 18-49 years
- Comparison of the HA- specific CD4 T cell response in adults receiving pure HA, cell-derived or egg-derived split or subvirion vaccines

Secondary Objectives

- Comparison of the epitope specificity of B cells following vaccination with HA or subvirion vaccines

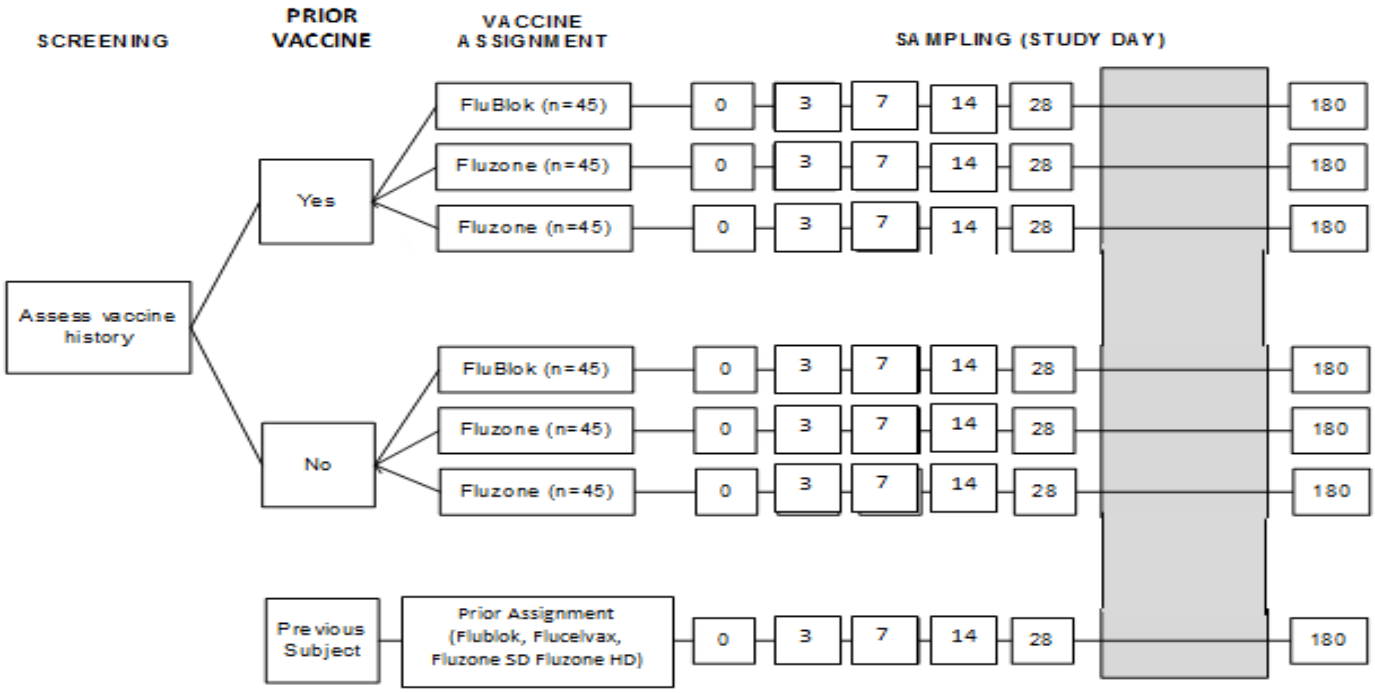
  Evaluation of the different vaccine formulations to induce HA specific antibodies that recognize circulating viruses

Tertiary Objective

- Confirmation of the safety of the licensed vaccines in healthy adults
-
- Evaluation of the effect of repeated vaccination on the immune response

Schematic of Study Design:

Because it is possible that prior exposure will impact the relative impact of peptide specific help on the antibody response, subjects will be screened for pre-vaccination antibody. Subjects will be divided into relatively high (greater than 1:8) or low (equal to or less than 1:8) pre-vaccination antibody based on pH1N1. The basic study design for the healthy adults age 18-49 years is described below. Subjects will be queried regarding prior history of influenza vaccination, and stratified into those who have not been vaccinated in the previous year and those who have, followed by randomized assignment to receive one of four licensed vaccines. Study subjects who participated in a previous year can be re-enrolled and will receive the same vaccine they received previously. Because the goal of the protocol is to evaluate the immune response to the vaccine and is not intended as a formal comparison of the adverse event profile, the vaccine assignment is not blinded. Following vaccination, subjects will have samples of blood obtained at the time points illustrated above. The last study visit will take place approximately 6 months after vaccination in order to test the durability of the response.



2 KEY ROLES

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3 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

3.1 Background Information

Inactivated influenza vaccines (IIVs) have been in use in the US and worldwide for the prevention of illness due to seasonal and pandemic influenza since their licensure approximately 60 years ago. Multiple studies over many years have evaluated the immunogenicity of these vaccines in various populations. In general, serum antibody responses to IIV are vigorous in healthy young adults with low levels of pre-vaccination antibody. Nasal antibody responses can also be detected, although they are not as vigorous as those seen after live vaccination.

The serum antibody response to IIV is impacted by several factors, including the age of the subject, the presence of chronic diseases, and the use of immune modulating drugs. In addition, the prior exposure of the subject to influenza vaccine or infection appears to have a substantial impact on both the immune response as well as the protective efficacy of IIV. For seasonal influenza vaccine, the magnitude of the serum antibody response is lower in individuals who begin with higher prevaccination titers, and highest in those with low prevaccination titers. Consistent with this observation, individuals with prior vaccination, and who therefore have higher baseline antibody titers, tend to have less vigorous antibody responses to revaccination. The impact of prior vaccination on protection is controversial. Some studies have suggested that vaccine efficacy is higher in those who were not vaccinated in the previous season than in those who were [1, 2]. Other studies have not seen this association [3]. The mechanisms by which prior exposure to vaccine or infection might modify the subsequent response to IIV are not known, but would have obvious implications for the design of new influenza vaccines and vaccine strategies.

Growth of virus in embryonated hen’s eggs has been used for production of IIV since these vaccines were first introduced. For the influenza A components of the vaccine, the selected reference antigenic variant for that season is typically reassorted with the high-yield laboratory influenza A/PR-8 virus to generate a seed virus that can replicate to high titer in eggs. In some cases, the seed virus may require additional passage in eggs to achieve adequate growth levels in this substrate. Harvested virions are subsequently concentrated, chemically inactivated, and disrupted with detergent, and the HA and NA proteins are partially purified by chromatography and other proprietary methods to yield a preparation referred to as split-product, subvirion, or subunit vaccine, depending on the manufacturer. In addition to containing a standardized amount of HA protein as determined by single radial immunodiffusion (SRID), licensed IIV preparations contain variable, but substantial amounts of NA as well as matrix (M) and

nucleoprotein (NP). Thus, it is possible that repeated exposure to the highly conserved NP and M proteins plays a role in the effect of prior vaccination on subsequent antibody responses.

Recently, two inactivated vaccines made from alternative substrates have been licensed in the US for seasonal use. These include mammalian cell culture grown inactivated vaccine (FluCelVax) [4] licensed for individuals ages 4 and above, and a purified recombinant baculovirus-expressed HA vaccine (FluBlok) made in insect cells [5] and licensed for ages 18 and above. Cell culture grown vaccine is made using a different type of seed virus and purification process which could result in a different mixture of proteins in the final preparation. In contrast, the baculovirus-expressed HA vaccine is a completely different preparation that contains over 95% pure HA protein without any other components of influenza virus. The availability of these additional licensed forms of influenza vaccine has provided an opportunity to evaluate the role that prior immunity to the various components of the vaccine plays in the antibody response, and to specifically address the hypothesis that CD4 recognition of epitopes specifically on the HA protein plays the major role in help for HA-specific B cell responses.

The basic composition of vaccines currently licensed in the US for seasonal influenza that are being used in this study are shown below:

Vaccine	Fluzone (SD)	Fluzone (HD)	Flublok	FluCelVax
Production	Eggs (avian)	Eggs (avian)	Sf9 cells (insect)	MDCK cells (mammalian
Inactivation	Formaldehyde	Formaldehyde	NA	b prioprolactone
Disruption	Triton X-100	Triton X-100	NA	Cetyltrimethyl-ammonium bromide
Purification	Sucrose gradient, additional concentration	Sucrose gradient, additional concentration	Column chromatography	ND
Dose	15 ug/HA	60 ug/HA	45 ug/HA	15 ug/HA
Indication	6 mo, and older	65 yrs and older	18 yrs and older	4 yrs and older
Components§				
H1N1	A/Michigan/45/2015 X-275*	A/Michigan/45/2015 X-275*	A/Michigan/45/2015	A/Singapore/GP1908/2015 IVR-180**
H3N2	A/Hong Kong/4801/2014 X-263-B*	A/Hong Kong/4801/2014 X-263-B*	A/Hong Kong/4801/2014	A/Singapore/GP2050/2015
B Yamagata	B/Phuket/3073/2013	NA	B/Phuket/307/2013	B/Utah/9/2014
B Victoria	B/Brisbane/60/2008	B/Brisbane/60/2008	B/Brisbane/60/2008	B/Hong Kong/259/2010

Source: Individual 2017-2018 FDA package inserts

§ All virus components are antigenically consistent with current WHO recommendations, however egg-grown viruses may contain egg adaptation changes not contained in mammalian cell or baculovirus expressed vaccines

*X-275 and X-263-B represent reassortants with A/PR/8 (H1N1) for high yield growth in eggs, the HA and NA are derived from the vaccine viruses

**IVR-180 represents a reassortant with A/Texas/1/77 (H3N2) selected for high-yield growth in mammalian cell culture, the HA and NA are derived from the vaccine virus

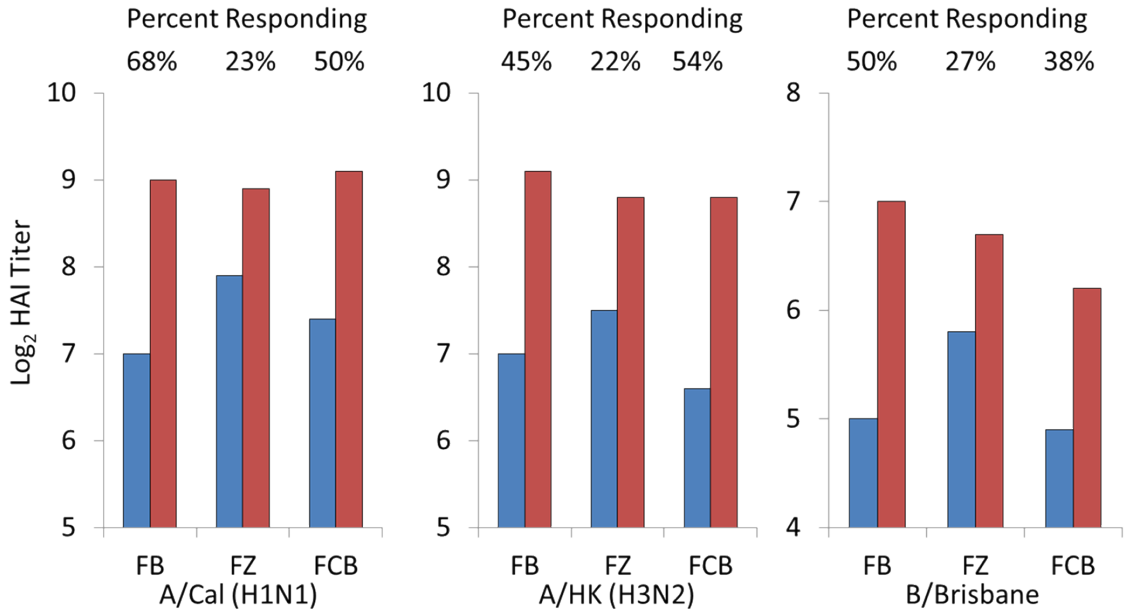
NA – Not applicable. ND – Not described in the package insert

3.2 Preliminary Data

We have previously demonstrated a wide breadth of CD4 repertoire to influenza, which includes up to 100 distinct peptides specificities in the primary response [6-8]. However, different populations of CD4 T cells, distinguished by their antigen specificity, may not contribute to the all functions important in development of a specific immune response. In particular, it has been demonstrated that protein vaccines and some enveloped viruses [9], delivery of help for antibody responses requires that the B cell and CD4 T cell recognize the epitopes from the

same protein antigen. In the case of influenza antibodies specific for HA and NA, CD4 T cells specific for epitopes in these proteins may be the only ones relevant for development of the antibody response. This help for high affinity Ab production may be primarily provided by cells belonging to the Tfh lineage (reviewed in [10]). However, most studies of the cellular immune response to influenza vaccine have not separately tracked the specific CD4 cells with these combined properties, leaving the contribution of these cells undetected. In contrast, monitoring CD4 T cells that do not have these specificities or function will likely contribute noise to the assessment of CD4 help for Ab responses to influenza.

In the first full year of this study, we found that there were unexpected differences in the serum antibody responses to vaccination between the study groups. In particular, serum HAI antibody responses to the baculovirus-expressed HA were more frequent and the mean fold rise was greater than those to the egg-grown vaccine, possibly consistent with the hypothesis that a more directed CD4 T cell response is more effective at providing help for antibody.



Interpretation of these results is complicated by the fact that the dosage level of the rHA vaccine was approximately 3 times that of the egg or cell culture vaccines, so that the more vigorous antibody responses may simply be dose related rather than reflective of CD4 specificity. Despite randomization, pre-vaccination titers were highest in the Fluzone group, as well as history of prior vaccination (18/22 in Fluzone, 16/22 in Flublok, and 13/26 in Flucelvax). In addition, we were unable to find significant numbers of subjects with pre-vaccination serum antibody titers of <= 1:8 to the pH1N1 component of the vaccines.

For these reasons, we modified our protocol by :

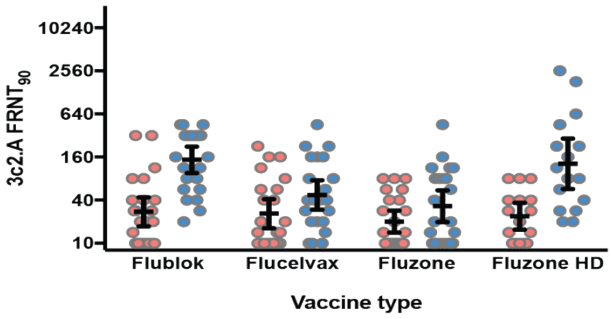
1. Adding the high dose influenza vaccine (Fluzone HD). This may allow separation of the effects of dose from the effects of vaccine composition

2. Eliminating screening for low titers of antibody to pH1N1 and instead stratifying randomization based on history of prior vaccination

3. Continuing to encourage re-enrollment in subsequent years with assignment to the same group, in order to assess the effect of vaccine composition on the response to revaccination.

Secondly, in the 2017-2018 we noted that the Flucelvax H3 component was as poorly immunogenic as that of Fluzone SD despite the differences in manufacturing (cell-based derivation versus egg-based). Post-vaccination antibody titers in Flucelvax recipients were low to all H3N2 viruses tested, including the egg-based, cell-based and two wild-type H3N2 strains. Recent findings have demonstrated that egg adaptation of vaccine viruses during vaccine development for products made in eggs has led to decreased protection against wild-type H3N2 viruses for products like Fluzone SD. However, we would have expected Flucelvax which avoids issues of egg adaptation, since vaccine viruses are grown in cell culture, to be more immunogenic to wild-type H3N2 strains. The reason for our findings of poor immunogenicity of the Flucelvax H3 components which was similar to egg-based Fluzone SD remains unclear and further study is needed outside the scope of the current project.

A



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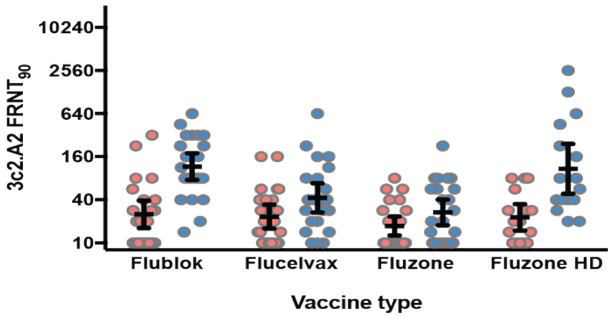


For this reason, though we have now chosen to eliminate the Flucelvax arm of the study for newly enrolled subjects. However, to preserve the ability to assess the secondary aim of the effect of repeated vaccination without introducing confounders, subjects who have received 2 or more consecutive years of Flucelvax will continue to receive the same vaccine in subsequent years.

3.3 Rationale

Our observations to date lead to the following model: Traditional egg-derived TIV have contaminating internal virion proteins that preferentially elicit memory CD4 T cells specific for these proteins. These CD4 T cells will have limited efficacy as helpers for the neutralizing Ab response and will suppress the CD4 T cell response to new HA epitopes in the vaccine. The current study will test this hypothesis by comparing CD4 cell responses to specific epitopes, and the subsequent B cell and antibody response, in subjects receiving a vaccine containing only HA protein to vaccines with more complex antigenic characteristics. The CD4 T cell reactivity to pools of unique, conserved, and total pH1 HA peptides as well as H3, influenza B HA, NP, and M1 peptides will be quantified using cytokine Elispot assays and flow cytometry, and then compared to the subsequent antibody and B cell response.

We will also use this study as an opportunity to evaluate the effects of prior vaccination. Recent studies have emphasized the potential negative effect of vaccination in prior years on both the immune response as well as the protective effectiveness of current vaccine. In order to evaluate this phenomenon in the context of multiple vaccine formulations, we will ascertain the prior vaccination history of the subjects and stratify vaccination based on vaccine history.

In addition, subjects who participated in this study in a previous year are eligible to re-enroll, and will receive the same vaccine that they were randomized to previously. This will allow an evaluation of differences between vaccine formulations in the responsiveness to multiple vaccinations.

Furthermore, recent studies indicate reports that the glycosylation pattern of viral hemagglutinins produced in cell culture can vary depending on the host cell used [11, 12], and that this can affect CD4 T cell immunogenicity and antibody recognition. As a cell culture-based influenza vaccine production platform offers many advantages and may eventually supplant the traditional egg-based approach, it is of great value to understand the CD4 T cell response induced by this vaccine and how this affects neutralizing Ab production.

Recent data have also suggested that the failure of seasonal influenza infection to induce substantial levels of stalk specific antibody may be due to the relatively inaccessible nature of this epitope. As part of this study, we will also compare the specificity of the human antibody response between the vaccine groups, with the hypothesis that the rHA vaccine will more readily allow targeting of these important, broadly conserved epitopes. We have compelling preliminary data demonstrating that multiple antibodies that we have isolated have a particularly slow on rate when they bind to the HA-stalk versus the HA-globular head epitopes on whole virions, but not on recombinant HA trimmers expressed in baculovirus. We hypothesize that a free, recombinant HA vaccine will allow more efficient targeting of the HA-Stalk epitopes.

3.4 Potential Risks and Benefits

3.4.1 Potential Risks

The risks and discomforts of this study include risks associated with the vaccine, the risks associated with study procedures (blood drawing and possible loss of confidentiality.

In placebo-controlled trials in adults, inactivated seasonal vaccines are associated with mild local pain at the site of administration. Although high-dose vaccine is not indicated in individuals under 65 years of age, clinical trials of such vaccines in healthy adults suggest that high dose vaccine is association with slight increases in mild local reactogenicity but are otherwise well tolerated [13]. Systemic symptoms like fever and malaise occur at rates equal to placebo.

During the swine influenza vaccine campaign of 1976, about 10 per 1,000,000 vaccine recipients in excess of the background rate developed the paralytic illness called Guillain-Barré Syndrome (GBS). In the subsequent decade, no association between seasonal influenza vaccine and GBS was found. More extensive investigations of this potential association occurring in the 1990s revealed that there was a small but detectable risk of GBS in the 6 weeks following seasonal influenza immunization: an attributable risk of approximately 1 per 1,000,000, adjusted for potential confounders. In the period since the Vaccine Adverse Event Reporting System (VAERS) was established in 1990, the rates of GBS reports following influenza vaccination have declined substantially. The annual reporting rate in that period was highest in the 1993-1994 influenza season (1.7 per 1,000,000 vaccinees) and lowest in the last season analyzed in the report, 2002-2003 (0.4 per 1,000,000 vaccinees) [14]. No cases of GBS have been reported following receipt of novel H1N1 influenza vaccines to date, but the total number of recipients is only in the thousands. Most persons who develop GBS recover completely.

Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur but can be prevented or mitigated by applying direct pressure to the draw site for several minutes. The use of alcohol swabbing and sterile equipment will make infection less likely at the site where blood will be drawn.

Personal health information of the subjects will be collected to determine eligibility and to evaluate safety outcomes throughout the study. Research personnel will make every effort to keep this information confidential. Still, a risk of participation is that the confidentiality of this information could be lost.

3.4.2 Known Potential Benefits

All four products being used in this study are licensed vaccines for seasonal influenza and would be expected to provide some protection against influenza illness caused by the strains contained within the vaccine.

4 OBJECTIVES AND OUTCOME MEASURES

4.1 Study Objectives

Primary Objective

- Comparison of the serum antibody response to immunization with pure HA, cell-derived and egg derived subvirion vaccines in adults age 18-49 years

- Comparison of the HA- specific CD4 T cell response in adults receiving pure HA, cell-derived vaccine egg-derived split or subvirion vaccines

Secondary Objectives

- Comparison of the epitope specificity of B cells following vaccination with HA or subvirion vaccines

  Evaluation of the different vaccine formulations to induce HA specific antibodies that recognize circulating viruses

Exploratory Objective

- Confirmation of the safety of the licensed vaccines in healthy adults
-
- Evaluation of the effect of repeated vaccination on the immune response

4.2 Outcome Measures

4.2.1 Primary Outcome Measures

The primary outcome measure for this study is:

- Mean change in the specificity, quantity and quality of serum antibody titers to the vaccine viruses, pH1N1, H3 and influenza B using HAI (year 1-5) and MN for pH1N1 and H3 viruses (years 1-5 from Day 0 to Day 28

- Mean change in the CD4 T cell reactivity to pools of total pHA peptides derived from the pH1N1 HA, as well as H3, influenza B HA, NP, and M1 peptides will be quantified using cytokine Elispot From Day 0 to Day 14

4.2.2 Secondary Outcome Measures

Secondary and tertiary outcome measures are listed below:

Secondary Outcome Measures

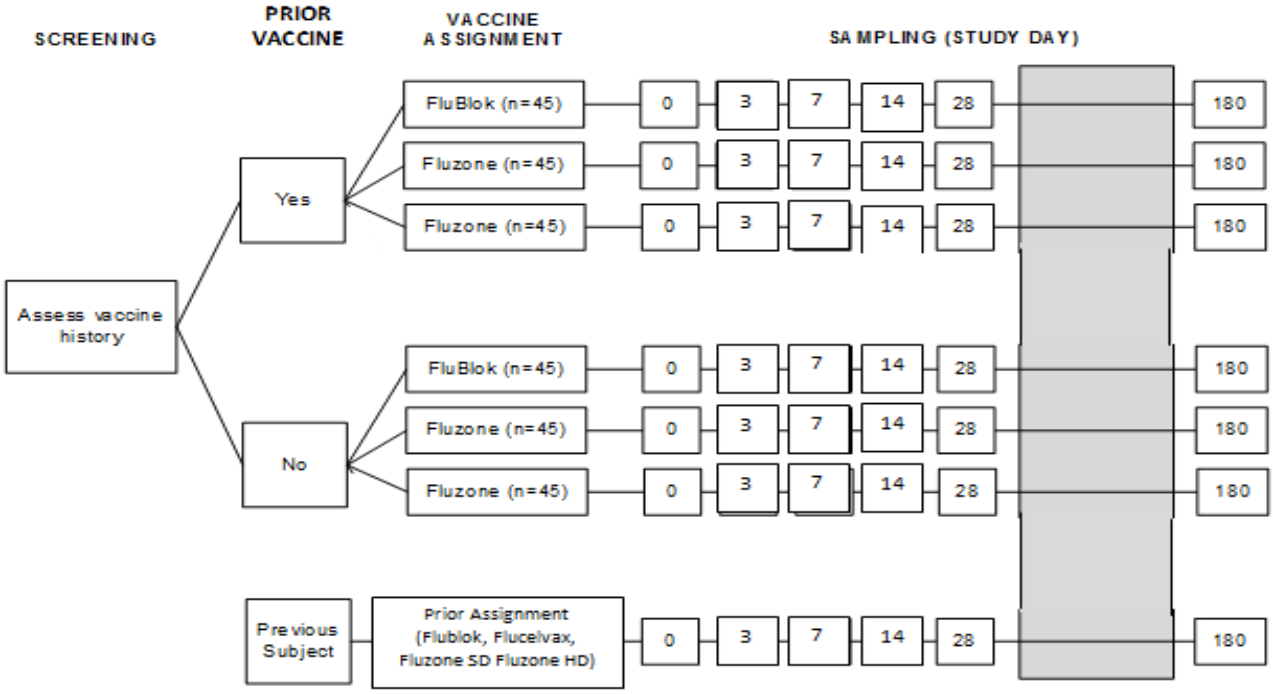
- Antibody specificity of cloned plasmablasts from subjects in each group
-
- Correlations of CD4 T cell ELISPOT number (Day 0 to Day 14) and antibody titers (Day 0 to Day 28) in subjects from each group (year 1-3), R value per cohort will be reported

Tertiary Outcome Measure

- Vaccine related adverse events
- Mean change in the CD4 T cell reactivity to pools of total pHA peptides derived from the pH1N1 HA, as well as H3, influenza B HA, NP, and M1 peptides will be quantified using cytokine Elispot from Day 0 to Day 14 for subjects with successive years of vaccination

5 STUDY DESIGN

Subjects age 18-49 years will be stratified by their prior vaccination history, followed by randomized assignment to receive one of three licensed vaccines: Fluzone SD, Fluzone HD and Flublok. Block randomization will be performed separately for the two groups. Study subjects who participated in a previous year can be re-enrolled and receive the same vaccine as previously (Fluzone SD, Fluzone HD, Flublok, Flucelvax). Because the goal of the protocol is to evaluate the immune response to the vaccine and is not intended as a formal comparison of the adverse event profile, the vaccine assignment is not blinded. Following vaccination, subjects will have blood sample obtained at the time points illustrated above. The last study visit will take place 6 months after vaccination.



6 STUDY POPULATION

Up to 500 subjects will be enrolled from the existing population of healthy adults residing in Rochester, NY and within the University of Rochester community, males and non-pregnant females aged 18-49 years, inclusive. Pregnancy status will be verified at screening and enrollment prior to vaccination. Women of childbearing potential will be asked to use a barrier method of birth control or an FDA approved form of birth control.

Study subjects will be recruited via placement of flyers in key locations on campus. Based on previous experience, we anticipate enrollment of 40 to 60 percent non-pregnant females in the study.

6.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to participate in this study:

1. Aged between 18 and 49 years of age (inclusive). (Participants who are returning for a subsequent year may be ≥ 50 years.)

2. Female subjects of childbearing potential must fulfill one of the following: (i) not able to bear children because she has been surgically sterilized (tubal ligation or hysterectomy) or (ii) agrees to practice effective methods of contraception that may include, but are not limited to abstinence, barrier methods, monogamous relationship with vasectomized partner, birth control pills, patches, hormonal shots or hormonal implants, NuvaRing and IUDs (intrauterine devices), from 30 days prior to study enrollment through 30 days following receipt of the last dose of vaccine.

3. Female subjects of childbearing potential must have a negative urine pregnancy test within 24 hours prior to vaccination.

4. Are in good health[1].

*[1]As determined by medical history and targeted physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days. This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to **improvement** of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on*

chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Topical, nasal, and inhaled medications (with the exception of inhaled corticosteroids as outlined in the Subject Exclusion Criteria (see Section 5.1.2), herbals, vitamins, and supplements are permitted.

5. Have normal vital signs (heart rate >55 to <100 bpm; blood pressure: systolic ≥ 90 mm Hg and ≤150 mm Hg; diastolic ≤ 90 mm Hg; oral temperature <100.0ºF);

6. The subject is able to understand and comply with the planned study procedures, including being available for all study visits.

7. The subject has provided informed consent prior to any study procedures.

6.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria at baseline cannot participate in the study:

1. Subject report of known hypersensitivity to allergy to components of the study vaccine or other components of the study vaccine.
2. Subject report of a history of severe reactions following previous immunization with licensed or unlicensed influenza virus vaccines.
3. Subject report of a history of Guillain-Barre syndrome within 6 weeks of receipt of a previous influenza vaccine.
4. The subject is a woman who is pregnant or breastfeeding or intends to become pregnant during the study period between enrollment and 30 days following receipt of vaccine.
5. The subject is immunosuppressed as a result of an underlying illness or treatment with immunosuppressive or cytotoxic drugs, or use of anticancer chemotherapy or radiation therapy within the preceding 36 months.
6. The subject received immunoglobulin or another blood product within the 3 months prior to enrollment in this study.
7. The subject has an active neoplastic disease (excluding non-melanoma skin cancer or prostate cancer that is stable in the absence of therapy) or a history of any hematological malignancy. For this criterion, "active" is defined as having received treatment within the past 5 years.
8. The subject has long-term (greater than 2 weeks) use of oral or parenteral steroids

9. The subject has received an inactivated vaccine within the 2 weeks or a live vaccine within the 4 weeks prior to enrollment in this study or plans to receive another vaccine within the next 28 days.
10. The subject has an acute or chronic medical condition that, in the opinion of the investigator or appropriate sub-investigator, would render vaccination unsafe or would interfere with the evaluation of responses. These conditions include any acute or chronic medical disease or conditions defined as persisting for 3 months (defines as 90 days) or longer, that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses of the subject's successful completion of the study.
11. Subjects with an active infection or that haves an acute illness or an oral temperature greater than 99.9F (37.7C) within 3 days prior to enrollment or vaccination. Subjects who had an acute illness that was treated symptoms resolved are eligible to enroll as long as treatment is completed and symptoms resolved > 3 days prior to enrollment.
12. The subject is currently participating or plans to participate in a study that involves an experimental agent (vaccine, drug, biologic, device, blood product, or medication) or has received an experimental agent within 1 month prior to enrollment in this study, or expects to receive another experimental agent during participation in this study, or intends to donate blood during the study period.
13. The subject has any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.
14. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others, within the past 10 years.
15. The subject has a diagnosis of schizophrenia, bi-polar disease, or other severe (disabling) chronic psychiatric diagnosis, or is receiving psychiatric drugs. Subjects who are receiving antidepressant drugs and are stable for at least 3 months prior to enrollment without decompensation are allowed enrollment into the study.
16. The subject has a history of alcohol or drug abuse in the 5 years prior to enrollment.
17. The subject has a known human immunodeficiency virus, hepatitis B, or hepatitis C infection.
18. The subject has any condition that the principal investigator (PI) believes may interfere with successful completion of the study.

6.3 Handling of Withdrawals

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after receiving the assigned vaccine will not be replaced. Participants who consented to the

study but did not receive the influenza vaccine will be replaced. These subjects will be considered as screen failures.

6.4 Termination of Study

Although the study sponsor has every intention of completing the study, the sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to internal safety review and recommendation, or at the discretion of DMID.

7 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

7.1 Study Product Description

Fluzone Standard Dose (Sanofi Pasteur). Quadrivalent subvirion vaccine (IIV4) produced in embryonated hen's eggs and administered intramuscularly at a dose of 15 ug of HA (as determined by SRID) per component.

Fluzone High Dose (Sanofi Pasteur). Trivalent subvirion vaccine (IIV3) produced in embryonated hen's eggs and administered intramuscularly at a dose of 60 ug of HA (as determined by SRID) per component.

FluCelVax (Novartis). Quadrivalent subunit vaccine produced in MDCK mammalian cell culture (ccIV4) administered intramuscularly at a dose of 15 ug of HA (determined by SRID) per component

FluBlok (Protein Sciences) Quadrivalent purified recombinant hemagglutinin vaccine (rIV4) produced in insect cells and administered intramuscularly at a dose of 45 ug of HA (determined by SRID) per component.

All four products are indicated for the prevention of influenza in adults, although Fluzone HD is only indicated for prevention in adults ages 65 and older.

7.1.1 Acquisition

All four vaccines will be ordered from the hospital pharmacy which will acquire the vaccine through distributors.

7.1.2 Formulation, Packaging, and Labeling

Fluzone HD and SD (Sanofi Pasteur)

Fluzone (Influenza Vaccine) for intramuscular injection is an inactivated influenza vaccine, prepared from influenza viruses propagated in embryonated chicken eggs. The virus-containing allantoic fluid is harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a non-ionic surfactant, octylphenol ethoxylate (Triton® X-100), 11 producing a "split virus". The split virus is further purified and then suspended in sodium 12 phosphate-buffered isotonic sodium chloride solution.

Fluzone is standardized according to United States Public Health Service requirements and is formulated to contain HA of each of the influenza strains recommended for the current influenza season. Vaccine is formulated in sodium phosphate-buffered isotonic sodium chloride solution containing formaldehyde, octylphenol ethoxylate and gelatin, in single dose prefilled syringes

Flucelvax (Sequiris)

FLUCELVAX (Influenza Vaccine), a vaccine for intramuscular injection, is a “subunit” influenza vaccine prepared from virus propagated in Madin Darby Canine Kidney (MDCK) cells, a continuous cell line. These cells were adapted to grow freely in suspension in culture medium. The virus is inactivated with ß-propiolactone, disrupted by the detergent cetyltrimethylammonium bromide and purified through several process steps. Each of the 3 virus strains is produced and purified separately then pooled to formulate the trivalent vaccine.

FLUCELVAX is a sterile, slightly opalescent suspension in phosphate buffered saline. FLUCELVAX is standardized according to United States Public Health Service requirements for the upcoming influenza season and is formulated to contain a total of 45 micrograms (mcg) hemagglutinin (HA) per 0.5 mL dose in the recommended ratio of 15 mcg HA of each of the three influenza strains recommended for that season/. Each dose of FLUCELVAX may contain residual amounts of MDCK cell protein (≤8.4 mcg), protein other than HA (≤ 120 mcg), MDCK cell DNA (≤ 10 ng), polysorbate 80 (≤ 1125 mcg), cetyltrimethlyammonium bromide (≤ 13.5 mcg), and β-propiolactone (<0.5 mcg), which are used in the manufacturing process.

FLUCELVAX will be supplied in single dose, pre-filled syringes. The vaccine contains no preservative or antibiotics, but the tip caps of the pre-filled syringes may contain natural rubber latex.

Flublok (Protein Sciences Corporation)

Flublok [15] is a sterile, clear, colorless solution of recombinant hemagglutinin (HA) proteins from three influenza viruses for intramuscular injection. It contains purified HA proteins produced in a continuous insect cell line (expresSF+®) that is derived from Sf9 cells of the fall armyworm, Spodoptera frugiperda, and grown in serum-free medium composed of chemically-defined lipids, vitamins, amino acids, and mineral salts. Each of the three HAs is expressed in this cell line using a baculovirus vector (Autographa californica nuclear polyhedrosis virus), extracted from the cells with Triton X-100 and further purified by column chromatography. The purified HAs are then blended and filled into single-dose vials.

Flublok is standardized according to United States Public Health Service (USPHS) requirements to contain 45 mcg of each HA of the strains recommended for that season.

A single 0.5 mL dose of Flublok contains sodium chloride (4.4 mg), monobasic sodium phosphate (0.195 mcg), dibasic sodium phosphate (1.3 mg), and polysorbate 20 (Tween®20) (27.5 mcg). Each 0.5 mL dose of Flublok may also contain residual amounts of baculovirus and

host cell proteins (≤ 28.5 mcg), baculovirus and cellular DNA (≤ 10 ng), and Triton X-100 (≤ 100 mcg).

Flublok contains no egg proteins, antibiotics, or preservatives. Flublok will be supplied in single dose vials, and the stoppers used for the single-dose vials are not made with natural rubber latex.

7.1.3 Product Storage and Stability

Influenza vaccines will be stored in secure, limited-access temperature monitored refrigerator environment at 2°C to 8°C (.6°F to 46°F) until needed. DO NOT FREEZE. The temperature of the storage unit will be monitored during the duration of the trial, and documentation of proper dedicated storage will be maintained. In the event of accidental deep-freezing or disruption of the cold chain, vaccines will not be administered; and the PI or the responsible person will contact the sponsor for further instructions.

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

Each of the vaccines used in this study will be formulated in single dose vials or syringes and will not require additional formulation prior to administration. A vaccine dose of 0.5mL will be administered by a clinical research nurse intramuscularly (IM) n the subject's preferred deltoid (upper arm) per the manufacturer's instructions.

7.3 Modification of Study Intervention/Investigational Product for a Participant

As there is only a single dose administered, there will be no dose or schedule modifications for any subject.

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

Study vaccine will be sent to the research site at the University of Rochester prior to the start of the study. Records of vaccine receipt and dispensation to the study subject as well as storage and destruction of the vaccine will be maintained according to existing standard operating procedures (SOPs)

7.5 Assessment of Participant Compliance with Study Intervention/Investigational Product

Not applicable, single intervention within the vaccine clinic only.

7.6 Concomitant Medications/Treatments

Administration of any medication or therapies considered necessary for the subject's welfare will be recorded and documented in the subject's source documentation. Concomitant medications will include all medications taken within 30 days prior to enrollment through 56 days post vaccination or early termination, whichever occurs first.

The following criteria will be reviewed with the subject's during each follow up visit. If any of these become applicable during the study, it will be noted in the subject's record.

1. Use of any investigational drug or investigational vaccine other than the study article.
2. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs (topical and nasal steroids are allowed).
3. Receipt of a licensed vaccine.
4. Receipt of immunoglobulins and/or any blood products.

8 STUDY SCHEDULE

8.1 Screening (Day -21 to Day 0)

Subjects will be screened for eligibility and for vaccine history prior to group assignment and vaccination. At the screening visit, the following procedures will be performed:

- The study will be explained to the subject, and the informed consent document will be signed. Subjects will keep a copy of the informed consent for their information.
- A medical history will be obtained, and details recorded in the case report form.
- A directed physical examination will be performed, if indicated by the medical history, directed at the relevant portions of the exam.
- The entry criteria will be reviewed and it will be verified that the subject meets all entry criteria. If the subject does not meet the entry criteria, the subject will be discontinued from the study, or can be rescreened in the case of entry criteria mandating temporary delays.

8.2 Vaccination (Defined as Day 0)

Subjects will be considered enrolled if they have signed the consent form. However, only subjects who met the entry criteria will be vaccinated and followed. Note that the screening visit and the vaccination visit may take place on the same day. At the vaccination visit the following procedures will be performed.

- An interim medical history will be obtained (unless the screening and vaccination visit are the same) including concomitant medications, detailing any subsequent medical events the subject has experienced since the screening visit.
- A directed physical exam will be performed, if indicated by the interval history, directed at the relevant portions of the exam.
- A urine pregnancy test will be obtained in female subjects. This test must be negative for the subject to continue in the study.
- The entry criteria will be reviewed with the subject and it will be verified that the subject continues to meet the entry criteria.

- A serum sample will be obtained from an arm vein for baseline antibody titers
- A sample of Peripheral Blood Mononuclear Cells (PBMC) will be obtained for assessment of baseline influenza-specific B cells and CD4 cells
- Subjects that were not vaccinated with influenza vaccine with the prior year's vaccine, will be randomized at the pharmacy into one of the three (FluBlok, Fluzone SD, Fluzone HD) vaccine groups, and will receive the indicated vaccine by intramuscular injection in open label fashion.
- Repeat subjects will receive the same vaccine they received previously. (FluBlok, Fluzone SD, Fluzone HD, Flucelvax)
- Subjects will be observed in the clinic for 30 minutes post vaccination for development of acute adverse events

8.3 Follow-up and Final Visits

8.3.1 Day 3 visit ((± 1 day)

Subjects will return 3 days after vaccination for the first visit. Because the timing of assessment of the cellular response to vaccination is critical, every effort will be made to ensure that subjects return on day 3. On the day 3 visit, the following procedures will be done:

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit
- If indicated by medical history, a directed physical exam will also be performed.
- Any adverse events (AE) will be recorded as described in the safety section 10.1 and appropriate corrective actions taken if indicated
- Peripheral Blood Mononuclear Cells (PBMC) will be collected from an arm vein for assessment of the CD4 T cell response to vaccination

8.3.2 Day 7 visit (± 1 day)

Subjects will return one week after vaccination for the day 7 visit. Because the timing of assessment of the cellular response to vaccination is critical, every effort will be made to ensure that subjects return on day 7. On the day 7 visit, the following procedures will be done:

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit
- If indicated by medical history, a directed physical exam will also be performed.
- Any adverse events (AE) will be recorded as described in the safety section 10.1 and appropriate corrective actions taken if indicated
- Peripheral Blood Mononuclear Cells (PBMC) will be collected from an arm vein for assessment of the B cell and CD4 T cell response to vaccination

8.3.3 Day 14 visit (± 1 day)

Subjects will return for follow up approximately 14 days after vaccination for assessment of the immune response. At that visit, the following procedures will be performed.

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit. If indicated by history, a relevant physical exam will also be performed.
- Any adverse events (AE) will be recorded as described in the safety section 10.1 and appropriate corrective actions taken if indicated
- Peripheral Blood Mononuclear Cells (PBMC) will be collected from an arm vein for assessment of the CD4 T cell response to vaccination

8.3.4 Day 28 visit (± 2 days)

Subjects will return for follow up approximately 28 days after vaccination for assessment of the immune response. At that visit, the following procedures will be performed:

- Interval medical history will be obtained including concomitant medications, reviewing any new events since the last visit

- If indicated by medical history, a directed physical exam will also be performed.
- Any adverse events (AE) will be recorded as described in the safety section 10.1 and appropriate corrective actions taken if indicated
- A serum sample will be obtained from an arm vein for antibody titers
- Peripheral Blood Mononuclear Cells (PBMC) will be collected from an arm vein for assessment of the B cell response to vaccination

8.3.5 Influenza surveillance

Influenza season will be defined as when community laboratories detect 4 or more positives in any one week for two weeks in a row, and will end when influenza is no longer detected. During influenza season, subjects will be asked to record weekly on a mobile device application (MyCAP) if they have developed influenza like symptoms.

MyCAP is a mobile app that interfaces with REDCap using REDCap's secure API. It gives researchers the ability to collect task oriented data on participants via the participants' mobile device in real time. Enrolling the participant and subsequently communication between the MyCAP mobile app and REDCap server is made possible by generating a unique QR code for each participant from the REDCap-MyCAP web portal. The mobile app scans this QR code which contains the project name, site and uniquely auto-generated participant id. The participant id is created by REDCap and is not based upon any data entered about the participant at any time or linked to any personal health information or identifying information. The mobile app and consequently the participant, only have the capability of submitting data to the project, eliminating the concern of data breaches since there is no capability to view or read any data from the project. The app will also have the capability to be password protected.

Once the mobile app has registered the QR code, participants will be asked to answer a weekly survey which they will be prompted to complete every Tuesday pertaining to the development of influenza like illness and associated symptoms. Data collected from participants in the mobile application will only be available to study staff. The following questions will be asked:

1) Have you had a fever or felt feverish in the past 5 days?

○ yes ○ no

2) Have you had any of the following symptoms in the past 5 days: runny nose, cough, sore throat, body aches?

○ yes ○ no

Subjects who do not have a mobile device compatible with the application or are unable or unwilling to utilize the application will be called weekly by the study staff and queried using the aforementioned questions. The app will send a reminder to study subjects at on Tuesday morning of every week to complete the surveillance questions. While there is no window built into the app for subjects to complete these questions it will be expected that they respond to the questions within 24 hours. Questions can only be answered once a week. If questions were not completed for the week the study team will be notified and this will be reviewed on Monday of the following week. If a subject misses two consecutive surveillance time points, the study team will call them to determine if there is an issue with the app or alternatively if they would prefer phone surveillances be conducted. If it is the latter case, the app will be uninstalled and weekly phone surveillance will be initiated. Three attempts will be made to contact subjects by phone and one attempt with a letter mailed to their street address. Subjects who cannot be reached by this method will be considered lost to follow up.

Subjects who report symptoms meeting the case definition of influenza (fever or feverishness plus either cough, rhinitis, or sore throat on the same or consecutive days) will be asked to return for an acute illness visit. At this visit the following procedures will take place

- Interval medical history will be obtained, including concomitant medications, reviewing any new events since the last visit.
- The presence and severity of respiratory symptoms will be assessed and recorded using a visual analog scale.
- A combined nasal and throat swab will be obtained for rtRT-PCR detection of influenza virus.
- If positive, the subject will be invited to participate in the Acute Flu study (DMID 14-0101) to assess the immune response to infection

8.3.6 Final study visit (Day 180 +/- 14 days)

Subjects will return approximately 180 days after vaccination for follow-up. This visit will be the study termination visit. At the final visit the following procedures will take place:

- Interval medical history will be obtained, including concomitant medications, reviewing any new events since the last visit
- If indicated by medical history, a directed physical exam will also be performed.
- Adverse events (AE) will be recorded and corrective actions taken if indicated

- A serum sample will be obtained from an arm vein for antibody titers
- Peripheral Blood Mononuclear Cells (PBMC) will be collected from an arm vein for assessment of the CD4 T cell response to vaccination

8.4 Early Termination Visit

If subjects discontinue from the study, they will be asked to make an early termination visit. At the time of the early termination visit, the reason for early termination will be recorded, current health status since the last visit will be reviewed, and all concomitant medications will be recorded. A targeted physical examination may be performed, as indicated, and information regarding AEs as described in the safety section 10.1 will be recorded.

Subjects will be encouraged to permit continued follow-up of AEs and to donate scheduled blood samples, if possible.

8.5 Unscheduled Visit

Unscheduled visits may occur at any time during the study. Any of the following activities may be performed:

- Medical history will be reviewed including concomitant medications, and updated as appropriate.
- All concomitant medications taken since the study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Information regarding AEs as described in the safety section 10.1 will be recorded. .
- Depending on the reason for the unscheduled visit, vital signs, including oral or axillary temperature, pulse, and blood pressure, may be obtained.
- If indicated by medical history, a directed physical examination relevant to the interval medical history may be performed.
- Additional laboratory tests may be obtained depending on the nature of the unscheduled visit.

9 STUDY PROCEDURES AND EVALUATIONS

9.1 Clinical Evaluations

Medical History: Study personnel will take the medical history of all subjects. This history will include significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. It will also include a history of allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease.

Medication History: Study personnel will record all medications, including prescription and over-the-counter drugs (such as vitamins, minerals, supplements, homeopathic preparations and/or therapies), taken by the subject in the 30 days prior to enrollment through 28 days after vaccination or early termination, whichever occurs first.

Medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications in this category include, but are not limited to, glucocorticoids, i.e., oral, parenteral and high-dose inhaled steroids, and immunosuppressive or cytotoxic drugs. The administration of licensed vaccines should be delayed until 21 days after the study vaccine. Subjects should not receive experimental agents including vaccines for the duration of the study

Targeted Physical Examination: Licensed study clinicians (i.e., physician, physician's assistant, nurse practitioner) may conduct a targeted physical examination if indicated based on review of history for assessment of AEs that require VAERS reporting as described in section 10.1, or if necessary to assist in determining eligibility. All subjects will have vital signs (blood pressure, pulse, and oral temperature) and height and weight measured prior to vaccination.

9.2 Laboratory Evaluations/Assays

9.2.1 Immunogenicity Evaluations

Serum hemagglutination-inhibition (HAI). HAI will be performed in microtiter format using turkey RBCs and egg-grown, betapropriolactone-inactivated A/California/07/09 virus as antigen. The titer of antibody will be defined as the highest dilution resulting in complete inhibition of hemagglutination. Sera will be treated with receptor-destroying enzyme and heat inactivated prior to testing at an initial starting dilution of 1:4. Sera with no detectable HAI titer will be assigned a titer of 1:2 for calculation purposes.

Microneutralization (MN) assay: Sera will be tested by microtiter technique for neutralization of an A/California/07/09 x PR8 reassortant virus in MDCK cells. Viral growth will be determined by ELISA of the cells following fixation with methanol using a combination of M- and NP-specific monoclonal antibodies. The titer of antibody will be defined as the highest titer resulting in 50% inhibition of antigen signal compared to un-neutralized control wells. Sera will be treated with RDE and heat inactivated prior to testing at an initial starting dilution of 1:10. Sera with no neutralizing titer will be assigned a value of 1:5 for calculation purposes.

B cell responses: Peripheral Blood Mononuclear Cells (PBMC) will be obtained before and on day 7 and 28 after vaccine and evaluated for B-cell responses. B cell responses will be evaluated by memory cell assay [16] and antibody secreting cell assay. In addition, B cells will be cultured in vitro and the functional quality of secreted antibody assessed by HAI and MN assays

CD4 T cell responses: PBMCs collected prior to and on day 3, 7, 14 and 180 after vaccination will be evaluated for the presence of antigen specific CD4 cells by ELISPOT assay and flow cytometry using a panel of peptides spanning the H1 hemagglutinin, neuraminidase and internal virion proteins such as NP and M1 [17]. This assay will identify both cross reactive and H1 specific epitope responses, as well as recall of memory cells to conserved internal virion proteins. PBMC will also be evaluated for the presence of antigen specific ELISPOT and cell homing markers by flow cytometry.

9.2.2 Specimen Collection, Preparation, Handling and Shipping

Instructions for specimen preparation, handling, and storage are included in the Manual of Procedures (MOP). Instructions for specimen shipment are included in the MOP.

9.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Urine or serum pregnancy tests will be performed within 24 hours prior to vaccination on all female subjects of childbearing potential (see above). Subjects with positive pregnancy tests are not eligible to participate. No other screening or clinical laboratory tests will be routinely obtained.

10 ASSESSMENT OF SAFETY

10.1 Specification of Safety Parameters

Only FDA approved, licensed seasonal inactivated influenza vaccine will be administered in this protocol as standard of care during influenza vaccine season, following the manufacturers' instructions and safety precautions. No serious adverse events are anticipated to occur to study participants as a result of this protocol.

Adverse events (AEs) related to administration of influenza vaccine include (as per Package Insert) injection site reactions (pain, swelling and erythema) and systemic reactions (headache body ache and muscle weakness). Since immunization with influenza vaccine is offered as standard of care to healthy adults, these anticipated reactions to the vaccine will not be considered reportable adverse events. Events meeting VAERS reporting requirements will be reported as indicated below.

There are minimal risks associated with venipuncture for obtaining research related labs. Foreseeable adverse events associated with venipuncture are: mild, temporary discomfort at the venipuncture site, bruising, and phlebitis. Very rarely, patients may experience vaso-vagal syndrome during phlebotomy. Vaso-vagal reactions may include diaphoresis, nausea, syncope and rarely loss of consciousness. These events will not be collected. However, severe vaso-vagal reaction, including loss of consciousness or > grade 2 hypotension will be reported as adverse events of special interest.

Any other adverse events that meet the reporting requirements of the Institutional Review Board will also be reported to DMID Medical Officer and the Clinical Project Manager.

10.2 Safety Reporting: VAERS and Adverse Events of Special Interest (AESI)

The investigator must report the following events to VAERS with a copy to the DMID Medical Officer, and the Clinical Project Manager will be notified by email, including a copy of the report (VAERS form). In addition, these events will be reported on the VAERS/AESI case report form:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine (Severe allergic reaction: e.g. (Anaphylaxis or anaphylactic shock 7 days post-vaccination) encephalopathy or encephalitis: e.g. coma, decreased level of consciousness, prolonged seizures within 7days of vaccine administration, Gullian-Barre syndrome within 6 weeks of vaccination, Arthus-type hypersensitivity reaction),

- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination found using the following link: https://vaers.hhs.gov/docs/VAERS_Table_of_Reportable Events_Following_Vaccination.pdf.
- Brachial neuritis (28 days)
- Any acute complications or sequelae (including death) of above events that occurs within the specified time period after vaccination,
- In the judgment of the PI, any unexpected reaction to the vaccine will be reported to VAERS as well as the DMID Medical Officer and the Clinical Project Manager.

The investigator must also report the following adverse events of special interest (AESI) on the VAERS/AESI case report form, and to DMID CPM and DMID Medical Officer. However, these events do not meet criteria for VAERS reporting:

- Severe vaso-vagal reaction following venipuncture, including loss of consciousness or > grade 2 hypotension.
- Infection or inability to breast feed following the use of the breast pump.

All adverse events that meet the reporting requirements of the Institutional Review Board will also be reported to DMID Medical Officer and the Clinical Project Manager.

10.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Urine pregnancy tests will be performed within 24 hours prior to vaccination on all female subjects of childbearing potential (see above). No other screening or clinical laboratory tests will be routinely obtained.

10.4 Reporting Procedures

ICH GCP 6, Section 4.11 require that an investigator notify the sponsor, regulatory authority(ies) and the local IRB immediately of any serious adverse event, deaths, or life-threatening problems that occur in the study. Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported to the IRB in accordance with reporting requirements specified by the IRB. Line lists of all adverse events and/or laboratory abnormalities will be reported via email to the DMID Clinical Project Manager and Medical Officer on a monthly basis.

10.5 Other Adverse Events (if applicable)

Non serious adverse events will be reported with the annual report for the study

10.6 Reporting of Pregnancy

Pregnancy events will be reported with the annual report of the study. All pregnancies will be followed to termination and the result of the pregnancy will be recorded in the study record.

10.7 Type and Duration of Follow-up of Participants after Adverse Events

All adverse events and serious adverse events will be followed until resolved, return to baseline or stable as determined by the study team.

11 CLINICAL MONITORING

Purpose: to protect the rights and well-being of human subjects in this study; to ensure that data are accurate, complete and verifiable from source documents; to ensure that conduct is in compliance with the currently approved protocol/amendments, with Good Clinical Practice, and with regulatory requirements.

11.1 Site Monitoring Plan

Site monitoring will be conducted using the DMID tools provided to ensure that human subject protection, study procedures, laboratory procedures, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as defined in the CQMP.

A protocol-specific Clinical Quality Management Plan (CQMP) has been approved for this study by DMID. The Quality Assurance (QA) plan will be implemented by a weekly review of source documents by the CRC to determine adherence to protocol requirements. The Quality Control (QC) plan will be implemented by daily observation and documentation of the site's work processes by study staff, to ensure that accepted procedures are followed.

Site visits may be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, sample tracking log, CRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions. The University of Rochester's IRB and other regulatory agencies may conduct study monitoring visits.

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12 STATISTICAL CONSIDERATIONS

12.1 Study Hypothesis

The primary hypothesis being tested in this study is that there will be differences in the serum antibody response, specificity, quantity and quality of memory B cell derived polyclonal antibodies (MpAb) and specificity and magnitude of the HA-specific CD4 T cell response between recipients of egg-derived and recombinant vaccines.

12.2 Sample Size Considerations

The power analysis is based on the primary aim of comparison of serum antibody, specificity, memory B cell derived polyclonal antibodies (MpAb) and HA-specific CD4 T cell counts (in log scale to stabilize variance) between the group of subjects vaccinated with egg derived TIV IM and the group of subjects vaccinated with Baculovirus derived TIV IM. In this explorative study, the HA-specific CD4 T cell response to the Baculovirus derived vaccine is unknown and thus no information is available about the group difference. But our past experience indicated the coefficient of variation (CV) of HA-specific CD4 T cell counts varied between 0.25 and 0.4 [18]. If we assume CV and use percent-change differences in HA-specific CD4 T cell counts relative to the group with egg derived vaccine, with 45 subjects per group, we will have 80% power to detect at least 10% ~ 20% difference in HA-specific CD4 T cell counts between the two groups. This power analysis is based on two-sample t test with a two-sided significance level of 0.05 and equal allocation of subjects to any two groups.

12.3 Planned Interim Analyses (if applicable)

No formal interim analysis is planned.

12.3.1 Safety Review

NA, no interim analysis of safety is planned.

12.3.2 Immunogenicity or Efficacy Review

NA, no interim analysis of efficacy is planned. Immunogenicity analysis will be done yearly as part of the overall analysis plan.

12.4 Final Analysis Plan

We will define the groups as: G1 (egg derived vaccine) = G1a (seronegative to pH1N1 virus and receiving egg derived vaccine) + G1b (seropositive and receiving egg derived vaccine); G2 (baculovirus derived vaccine) = G2a (seronegative to to pH1N1 virus and receiving baculovirus derived vaccine) + G2b (seropositive and receiving baculovirus derived vaccine). The log transformation will be applied to stabilize variance, as suggested by our past experience [18]. The between-group and among-group comparison in each of the outcomes will be based on both standard statistical analysis approaches using summary statistics and the complicated random variance model approach. Specifically, each of CD4 specific cells for different Influenza proteins (eg HA, NP etc), memory B cell derived polyclonal antibodies, HAI and MN titers among the four groups and between G1 and G2, between G1a and G2a, and between G1b and G2b, we will use maximum responses or maximum boost responses (maximum value after day 0 – value at day 0) or maximum fold increase as endpoint and do group comparison by ANOVA for among-group comparison and two-sample t-test for between-group comparison, or their nonparametric analogy of Kruskal-Wallis test and Wilcoxon Rank sum test, respectively, may also be applied for robustness. Multiple comparisons may subject to Bonferroni correction, if appropriate. Correlation test such as Pearson correlation analysis, or its nonparametric analogy of Spearman correlation analysis, will be used to study how baseline CD4 responses and/or memory B cell derived polyclonal antibodies, HAI, MN titers affect the boost responses of CD4, memory B cell derived polyclonal antibodies, HAI and MN titers after vaccination.

Various parametric or nonparametric mixed-effects regression approaches 99 will be used to study the dynamic curve of each outcome, to take account of the correlation among observations from the same subjects over time. Group indicator will enter the model as a predictor and thus can be compared simultaneous at each time point and also by difference from difference approach to control the effect of baseline values. Demographic information may be controlled by entering the model as predictors. To further investigate the effect of CD4 cell specificity in helping neutralizing antibody responses, regression analysis will be applied, with boost or fold increase of HAI or MN titers as responses, all the CD4 cell specificities as predictor, possibly adjusting demographic variables. Type I and III sum of squares and standard coefficient estimates can be used to compare the effect of CD4 specificities in helping neutralizing antibody responses. The parametric or nonparametric mixed-effects regression approaches may also be used to compare the effects to take account of within-subject correlation. Among-group plasmablast comparisons will be proceeded by: 1). One-way ANOVA, or its nonparametric analog of Kruskal-Wallis test; 2). Repeated ANOVA or its nonparametric analog of Friedman test for simultaneous pairwise comparison, with within-subject correlation taken into account.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this study in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. These representatives will be permitted access to all source data which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, x-rays, and subject files kept at the laboratories involved in the study. CRFs will serve as source documents. All study documents will be secured by key and/or password protection.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance.

The Principal Investigators will provide direct access to all study-related field sites, source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The Principal Investigators will ensure all study staff are appropriately trained and applicable documentations are maintained on site.

DMID reserves the right to review site CQMP findings.

The site will implement QC procedures with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be clarified and resolved by going back to the source documents and checking with the field team that collected the data.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 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), if applicable. The investigator's institution will hold a current Federal Wide Assurance (FWA) issued by the Office for Human Protections (OHRP) for federally funded research.

15.2 Institutional Review Board

The University of Rochester will provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. The UR IRB operates under U.S. Federal-Wide Assurance (FWA)

Prior to enrollment of subjects into this trial, the approved protocol and the informed consent form will be reviewed and approved by the appropriate IRB. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB and submitted to the sponsor. Notification of the IRB's composition, or the IRB's Federal-wide Assurance number, will be provided tp DMID.

Should amendments to the protocol be required, the amendments will be written by the PI for submission to the IRB and also submitted to the sponsor. The site will submit to the sponsor a copy of the IRB letter of approval of the amendment.

15.3 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. At the time the study worker will seek informed consent, the study worker will ask the eligible participant (or the participant's representative) if the participant is literate. If the eligible participant reports he or she is not literate, then the study worker will request that a witness be present while the study worker reads and explains the study and what participation will entail. If the eligible participant

accepts to take part in the study, he or she will make a mark on the signature line of the consent form. The witness will also sign and date the form, if the witness is confident that the participant has understood the explanation and is participating willingly. In addition, the witness will complete the date line for the participant.

Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants 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.

Subjects who participated in year 1 of the study will be allowed to participate again the following year. Subjects who agreed to future contact will be contacted by phone and/or letter to invite them to participate.

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

No minors will be enrolled in this study

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

Because not all of the vaccines being studied are licensed for use in individuals under 18, children will not be included in this study. Because pregnancy could alter the immune response to vaccination, pregnancy is an exclusion to participation. Otherwise there are no exclusions of special populations.

15.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological specimens and genetic tests in addition to the clinical information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. A representative from the University of Rochester IRB may also have access to the subject's record.

To further protect subject confidentially in the United States, research participants in NYICE protocols are protected by a Certificate of Confidentiality from Department of Health and Human Services (DHHS), which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form.

15.6 Study Discontinuation

If the study is discontinued, enrolled subjects will continue to be followed for safety assessments. No further doses of vaccine will be administered.

15.7 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining specimen for possible use in future research studies, such as testing for antibodies against other viruses or bacteria. Samples will be stored at the local site and will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality. Such testing may be performed by collaborating laboratories located at other sites.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records, but subject's samples may be kept with the study records or in other secure areas. Subjects can decide if they want their samples to be used for future research or have

their samples destroyed at the end of the study. A subject's decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

16 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data using black ink to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed out with a single line, initialed and dated. The original text will not be erased, overwritten, or altered with correction fluid or tape on the original.

16.1 Data Management Responsibilities

All source documents and laboratory reports will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete.

Data collection is the responsibility of the clinical study staff at the site under the supervision of the clinical PIs. During the study, the investigators will maintain complete and accurate documentation for the study.

16.2 Data Capture Methods

Clinical data will be initially recorded on paper source documents, and then transferred to electronic case report forms (eCRF) within BLISS. Source documents will be retained for monitoring purposes in a secure location. Laboratory data will be directly entered into BLISS from laboratory notebooks

As detailed in the CEIRS contract, overall CEIRS data sharing will adhere to the following schedule:

- Sequence data: provided to Data Processing Coordinating Center within 45 days
- Surveillance data: provided to Data Processing Coordinating Center within 12 months
- Virus phenotypic data: provided to Data Processing Coordinating Center within 12 months
- Basic research data: provided to Data Processing Coordinating Center within 2 months post publication.

Acute surveillance clinical data will be captured using the MyCAP personal device application which is password protected. This aplication will be uploaded onto the subjects' personal device using a unique QR code which is not linked to personal health information. The application interfaces directly with RedCap and data will be transmitted in real-time as it is entered. No data will be stored permanently on the subjects' personal devices.

16.3 Types of Data

Data for this study will include subject demographics, safety assessments including reactogenicity events after vaccination, and research laboratory results including antibody responses and measurements of B cell, CD4 cell, and transcriptional responses.

16.4 Timing/Reports

The final report will include a comprehensive analysis of the data.

16.5 Study Records Retention

Records and documents pertaining to the conduct of this clinical study, including CRFs, source documents, and consent forms must be retained by the investigator for at least 2 years following the date of completion of the study. No study records will be destroyed without prior authorization by DMID. These documents should be retained for a longer period, however, if required by local regulations.

16.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific MOP requirements or institution SOPs. The noncompliance may be either on the part of the subject, the site principal investigator, or other study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, Section 5.1.1
5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. A line listing of deviations will be reported to DMID on a monthly basis.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as a copy kept in the subject's source document file. Protocol deviations must be sent to the local IRB/IEC per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

17 PUBLICATION POLICY

Following completion of the study, the research investigators will share data as defined in the contract and as directed by the CO and COR.

18 LITERATURE REFERENCES

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19 SUPPLEMENT/APPENDICES

Table of study procedures

	Study Day (numbers denote blood volume in mL)							
Procedure	d-21 to 0	0	3	7	14	28	180	Day 0-56 Blood Volume
Informed Consent	X							
Medical History	X							
Interval History		X	X	X		X	X	0
Directed Physical Exam*		X	X	X	X	X	X	0
Urine Pregnancy Test**		X						0
Review Entry Criteria	X	X						0
Serum for antibody		10				20	10	30
PBMC for B cells		30		60		60		150
PBMC for CD4 T cells		100	50	80	50		50	280
Randomization		X						
Vaccination		X						
AE review			X	X		X	X	
Total		140	50	140	50	80	60	460
*Examination if indicated by history **Female subjects only								