Protocol Cover Page

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Analysis of Bone Marrow and Blood B cell Immune Responses to Influenza Vaccination

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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 clinical study 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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** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site.*

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List of Abbreviations

AE	Adverse Event
ASCs	Antibody Secreting Cells
CFR	Code of Federal Regulations
CEIRS	Center for Influenza Excellence in Research and Surveillance
CoC	Certificate of Confidentiality
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic CRF
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HA	Hemagglutinin
HHS	Health and Human Services
HI	Hemagglutination Inhibition
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IMGT	Immunogenetics Information System
IM	Intramuscular
IRB	Institutional Review Board
ml	Milliliter
MOP	Manual of Procedures
NA	Neuraminidase
NGS	Next Generation Sequencing
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
PHI	Protected Health Information
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
TIV	Trivalent Inactivated Vaccine
US	United States
VAERS	Vaccine Adverse Event Reporting System
WCI	Winship Cancer Institute
WNL	Within Normal Limits

Protocol Summary

Title: Analysis of Bone Marrow and Blood B cell Immune Responses to Influenza Vaccination

Population: Male and female individuals aged 18-49 years.

Number of Sites: 1

Study Duration: 4 years

Participant Duration: 365 days (1 year) – 1095 days (3 years)

Objectives:
Primary Objective: To investigate the longevity of humoral immunity to influenza virus in humans.

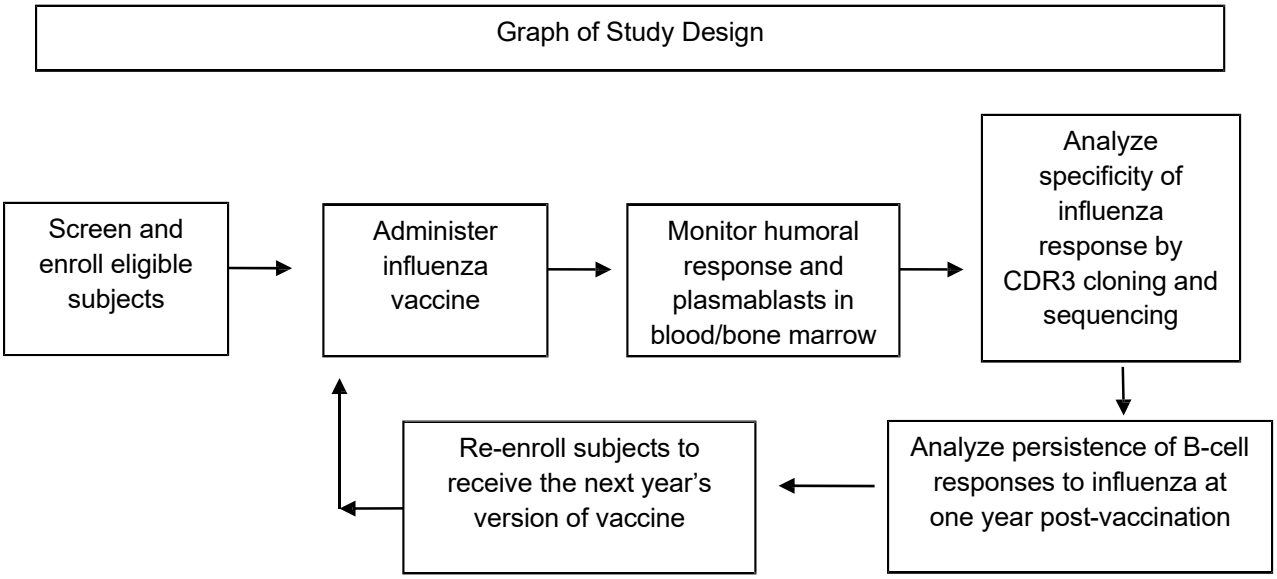
Secondary Objectives: Longitudinal tracking of vaccine-induced B cell responses with special emphasis on broadly neutralizing HA stem reactive responses.

Study Summary:
This is a Phase IV open label and single arm observational study enrolling up to 55 healthy males and non-pregnant females, 18-49 years old, inclusive. The study is designed to assess the humoral response to influenza vaccination and the longevity of humoral immunity to influenza vaccination in healthy adults. The laboratory technique will characterize persistence and clonotype of antigen specific B-cells and plasmablasts in blood and bone marrow. Enrolled subjects will receive licensed seasonal inactivated influenza vaccine (administered as a part of the study). The vaccine will be administered according to the package insert, and study participants will donate serial samples of blood and bone marrow aspirate for immunology monitoring. Safety will be assessed from the time of study enrollment through the last study visit, via monitoring of vital signs, change in health status, and targeted physical exam with safety labs prior to each bone marrow aspirate procedure. Repeated measurements of humoral immunity will be obtained at 7 days, 14 days, 28 days, 90 days and at one year post vaccination to assess the magnitude, clonal diversity and persistence of B-cell responses to influenza vaccination.

Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study. Follow-up visits were also halted, which impacted the timing of participants' subsequent follow-up visits. For this study, there are participants whose Day 28 and Day 90 visits were impacted by the temporary halting of non-essential research studies. We will request an expansion of the study window for the participants whose visits were impacted. Additional details regarding this are detailed further in the protocol. None of the other timepoint visits were impacted. Dependent upon Emory's guidelines in response to the evolving nature of the pandemic and the prioritization of studies being allowed to re-open, time will be

needed to secure the appropriate approvals and clearance to bring research participants back for their missed visits. As such, we would like to request that the missed visit windows for the Day 28 and Day 90 visits be extended for a maximum of up to 180 days, to ensure that ample time is available to bring participants back for their missed visits. Enrollment for this study ended on March 31, 2020.

Subjects will be offered the opportunity to participate in the study for up to 3 consecutive years provided eligibility criteria is met each year. Subjects who elect to continue in the study after first year of participation will be rescreened to verify continued eligibility and re-consented prior to subsequent participation. Re-enrolling subjects will receive new subject identifiers and will count towards the total enrollment number for subsequent years of participation. A separate subject record will be maintained each year a subject re-enrolls in the study.



Schematic of Study Design:

Subjects enrolled in study	**Years study performed**	**Number of subjects per year (study total)**	**Blood or Bone Marrow samples (days post-influenza vaccination)**
Healthy adults (18-49 years)	Years 1-4	Years 1-3: 15/year Year 4: 10 (Total: 55)	Blood volume 64ml per visit (320 ml total)– Day: 0, 7, 14, 28, 90, 365* Bone marrow aspirate volume 30 ml per visit (90ml total)– Day: 0, 28, 365*

* Subjects that consent to be in the study for subsequent years, the "Day 0" of the second and third years will constitute "day 365" and "day 730" with respect to the first year of participation.

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Annual influenza virus epidemics by subtype A viruses (H1N1 and H3N2), and/or influenza B viruses have been shown to cause substantial morbidity and mortality, exacting an average of 36,000 deaths and more than 200,000 hospitalizations in the US alone (1).

To combat this important public health threat, two types of inactivated influenza vaccines have been approved by the FDA: 1) a trivalent, inactivated vaccine (TIV); and 2) a quadrivalent inactivated vaccine (QIV). The TIV vaccine contains the hemagglutinin (HA) and neuraminidase (NA) proteins corresponding to the three influenza viruses predicted to be in circulation during the influenza season (two A strains and one B), and the quadrivalent type contains the HA and NA for an additional B strain. These vaccines induce predominantly HA-specific and NA-specific antibodies that are capable of virus neutralization. Antibodies to HA are critical to provide immune protection to the host against influenza virus infection, as this protein plays a key role in virus entry to initiate the replication cycle in the cell. A live attenuated vaccine has also been approved but this study will use only inactivated influenza vaccine.

Currently, the Advisory Committee on Immunization Practices of the CDC recommends that everyone aged 6 months or older in the United States receive the influenza vaccine on an annual basis (2). The recommendation for annual vaccination, regardless of previous vaccination history, is in part due to the constantly changing nature of the influenza strains in circulation. Through the processes of antigenic drift and antigenic shift, the viral HA protein is constantly changing to evade the antibody response. This requires that the formulation of the influenza vaccine must be updated each season. However, when the vaccine formulation remains unchanged from year to year, re-vaccination is still recommended because antibody levels decline over the course of a year (3).

In recent years it has become apparent that a significant fraction of the human antibody response to influenza targets highly conserved neutralizing epitopes on the virus (4). This raises the possibility that an optimally designed vaccine could elicit a broad immunity to the virus. If long-lived antibody responses could be generated against these neutralizing epitopes through immunization, it could eliminate the need for annual vaccination. The development of such a vaccine will therefore require an understanding of the factors that lead to the generation of long-lived antibody responses.

Previous data has shown that influenza vaccination results in the production of a robust ASC response in the blood (5). This ASC response is short-lived however and is composed of a dividing, migratory cell population that is thought to take up residence in specialized survival niches in order to produce antibodies over longer periods. Animal studies have indicated that the bone marrow is a major site for the long-term persistence of ASCs following vaccination or infection (6). This persistence has been shown to be true for humans as well (7). A close correlation has been observed between the numbers of influenza-specific ASCs in the bone marrow of human subjects and influenza specific antibody titers in their serum. An earlier

study demonstrated increases in influenza-specific ASCs in the bone marrow of these subjects following vaccination (manuscript in preparation, R. Ahmed, et. al). Furthermore, the magnitude of this increase in bone marrow ASC numbers correlated with the size of the blood ASC response. Individual B-cell clones that participated in the ASC response in blood samples obtained one-week post vaccination appeared in the bone marrow one month post-vaccination.

Preliminary research, based on a relatively limited number of donors from an earlier study, indicates that the majority of influenza-specific bone marrow ASCs initially generated after vaccination are lost within one year (manuscript in preparation, R. Ahmed, et. al). It was noted; however, that a subset of ASCs remain one year after vaccination, suggesting that it is possible to generate long-lived responses during influenza vaccination (manuscript in preparation, R. Ahmed, et. al). A critical unresolved question is whether ASCs, which produce broadly neutralizing antibody responses, are among these long-lived cells. In this study, state-of-the-art biological and molecular techniques will be used to track the persistence of influenza specific ASC responses over long periods. This study will characterize the magnitude of ASC responses targeting conserved vs. strain specific HA epitopes over the short- and long-term. Characterization of the antibodies that do or do not persist based on their target epitope, level of somatic hypermutation, affinity for antigen, and other factors will provide insight into the selection forces and differentiation pathways which give rise to truly long-lived responses. By understanding the factors that determine the longevity of a B-cell clone, there will be a better understanding of how to design a vaccine to preferentially induce long-lived ASCs.

2.2 Scientific Rationale

Although extensive serological assays, especially hemagglutinin inhibition (HI) titers, have been used as a marker for protective immunity, these assays have not provided the kind of insight that are critical for understanding the precise nature of vaccine-induced immunity in humans. As neutralizing antibodies are the main human correlate of protection from influenza infection it is vital to gain more insight into the kinetics and magnitude of the long-lived plasma cells present in the bone marrow.

Assessing human responses to influenza vaccines must be performed in humans.

- The results of preliminary research from the Ahmed group have shown that there is an increase in the number of influenza-specific plasma cells in the bone marrow one month after vaccination. In a previous study in healthy subjects, characterization of the plasmablast response, following influenza vaccination provided preliminary data on the longer-term persistence of influenza-specific antibodies and plasma cells. In the majority of these subjects, the bone marrow plasma cell numbers and serum anti-influenza titers returned to their pre-vaccination levels by one year after vaccination. This is in contrast to the longer-lasting antibody responses seen with other vaccines. In a minority of influenza-vaccinated subjects, plasma cell numbers and antibody titers were maintained at higher levels at one year after vaccination.

- Enrolled subjects will receive licensed inactivated influenza vaccine and undergo a bone marrow aspiration on days 30 and 365 (+/- 3 months) post-vaccination. Serial monitoring of the ASC in bone marrow will accurately define the lifespan of plasma cells induced by influenza virus. Several secondary objectives related to plasma cell longevity will be addressed:

  i. Identify characteristics of the acute immune response to vaccination (e.g. the magnitude of the initial plasmablast response) that are associated with maintaining bone marrow plasma cells and antibody levels at late time-points
  ii. Describe the persistence or loss of newly generated B-cell and influenza-specific plasma cells
  iii. Identify characteristics of antibody clones (e.g. degree of affinity maturation) that are associated with longer-term persistence in the bone marrow compartment.
  iv. Identify phenotypic differences between influenza specific plasma cells at one month post vaccination versus one year post vaccination.

  The answers to these questions will have important implications for designing vaccines to elicit longer-lived plasma cell responses.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The physical risk of drawing blood is local pain and bruising at the site of venipuncture. Qualified personnel will draw blood samples. Care will be taken to obtain these specimens in a safe and hygienic manner. A small number of people experience lightheadedness or fainting. There is a slight risk of infection. Repeated blood drawing may be associated with the development of anemia. To avoid this risk, no more than 385 ml of blood will be obtained over a period of one year.

The physical risks of bone marrow aspiration and local administration of lidocaine are pain and bruising that may last 1 to 3 days. Very rarely more serious side effects which include allergic reactions to lidocaine or damage to normal blood vessels, veins or bone structures or localized infection at the area where the marrow is removed. Reactions to lidocaine include cutaneous lesions, urticarial, edema or anaphylactoid reactions. Pruritis, burning, edema, erythema, purpura and bleeding may occur at the local injection site. The removal of 30 ml or less of marrow produces a temporary and mild anemia that may last one or two weeks. A small number of people experience vaso-vagal response including, lightheadedness, nausea, and transient hypotension. Bone marrow aspiration procedures will be performed by a board certified hematologist/oncologist. Care will be taken to obtain these specimens in a safe and sterile manner.

The side effects from inactivated influenza vaccine are generally mild. They include soreness at injection site (10-64% of subjects) that lasts <2 days. When the vaccine is given, the subject may feel a slight pain and burning during the injection. Fever, malaise and myalgia can occur after vaccination with inactivated influenza vaccine. These reactions begin 6-12 hours after

vaccination and can persist for 1-2 days. Allergic reactions rarely occur after influenza vaccination and are generally caused by residual egg proteins. Persons with a history of egg allergy will be excluded from study participation. Associated with the 1976 swine influenza vaccine, there is a slight risk of Guillain-Barre syndrome with the administration of the seasonal influenza inactivated influenza vaccine. Persons with a history of Guillain-Barre syndrome will be excluded from study participation.

Occasionally, recipients of unadjuvanted licensed, inactivated influenza virus vaccines may develop influenza-like reactions, such as fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), arthralgia (joint pain), headache, and/or nausea. Some subjects may develop reactions at the injection site, including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and/or tenderness. With unadjuvanted licensed, inactivated influenza virus vaccines most of these reactions peak in intensity in the first 24 hours after vaccination and usually disappear without treatment within 1 or 2 days. Analgesics (e.g., acetaminophen, or ibuprofen or similar non-steroidal anti-inflammatory drugs [NSAIDs]) and rest may generally relieve or lessen these reactions. Bruising can sometimes occur due to the vaccination procedure.

Acute and potentially life-threatening allergic reactions are also possible. Very rarely, occurring in about 1 in 4 million people given a vaccination, there can be a serious allergic reaction to a vaccine. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or loss of blood pressure (hypotension). If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur.

In addition, post-marketing surveillance indicates the following adverse events of special interest (AESI) as potential risks for the seasonal influenza vaccines: neuritis, convulsions, severe allergic reactions, syncope, encephalitis, thrombocytopenia, vasculitis, and Guillain-Barré syndrome. Reports of these reactions were rare; however, exact incidence rates cannot be precisely calculated.

During the swine influenza (H1N1) vaccine campaign of 1976, some recipients developed a paralytic illness called Guillain-Barré syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza (H1N1) vaccine at about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines. Most persons who develop GBS recover completely, although the recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack. Intensive surveillance of GBS after administration of inactivated influenza vaccines since 1976 has shown a slight increase in risk over background cases (more than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination (44). Interestingly, although

vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine-associated GBS have declined (45). A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older (46). An active, population-based surveillance study conducted during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines (47-49). Another study using the Medicare system showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48) (50). An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS (51). It is unknown if the administration of the inactivated A/H7N9 vaccine to be used in this study will result in the incidence of GBS that was seen with the 1976 vaccine product as the mechanism leading to this response has not been completely elucidated.

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU sites. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating sites for quality assurance and data analysis include groups, such as National Institute of Allergy and Infectious Diseases (NIAID) and Food and Drug Administration (FDA).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This web site will not include information that can identify subjects. At most, this web site will include a summary of the results.

There may be other unknown risks, discomforts, or side effects.

2.3.2 Known Potential Benefits

There is no benefit to subjects who undergo blood drawing or bone marrow aspiration. Seasonal influenza vaccine is considered beneficial to most subjects, as it generally provides protective immunity against the influenza strains within the vaccine. If vaccine is administered after flu season, there is no benefit to the subject. Data from this study may yield a better understanding of the current body of knowledge describing human infections with influenza viruses.

3 OBJECTIVES

3.1 Study Objectives

Primary Objective: To investigate the longevity of humoral immunity to influenza virus in humans.

Secondary Objectives: Longitudinal tracking of vaccine-induced B cell responses with special emphasis on broadly neutralizing HA stem-reactive responses.

3.2 Outcome Measures

3.2.1 Primary Outcome

Primary Objective Measures: To investigate the longevity of humoral immunity to influenza virus in humans by:

- Determination of the number of influenza-specific antibody secreting cells (ASC) present in the bone marrow prior to vaccination and at 28 days and one year post vaccination.

- Determination of the number of influenza-specific antibody secreting cells (ASC) present in the blood 7 days post-vaccination.

- Determination of the number of influenza-specific memory B cells present in the blood prior to vaccination and at 14 days, 28 days, 90 days, and 365 days post-vaccination.

- Determination of the percentage of subjects achieving seroconversion to each of the three strains contained in the trivalent vaccine. Seroconversion is defined as having a pre-vaccination hemagglutination inhibition (HAI) titer <1:40 against a particular strain and a day 28 post-vaccination titer ≥1:40 against the same strain.

- Determination of the percentage of seroconverting subjects who maintain an HAI titer ≥1:40 approximately 365 days after the study vaccination.

3.2.2 Secondary Outcome Measures

Secondary Objective Measures: Longitudinal tracking of vaccine-induced B cell responses with special emphasis on broadly neutralizing HA stem reactive responses by:

- Determination of the frequency of hemagglutinin stem reactive responses to the influenza vaccine among the blood plasmablasts at 7 days post-vaccination and among the bone marrow plasma cells at 28 and 365 days post-vaccination.

3.2.3 Exploratory Outcome Measures

Secondary Objective Measures: Longitudinal tracking of vaccine-induced B cell responses with special emphasis on broadly neutralizing HA stem reactive responses by:

- Determination of the dominant antibody families (clonotypes) induced by influenza vaccination.
    - Use single cell sorting and multiplex PCR to identify influenza specific clonotypes among the blood plasmablasts at day 7 post-vaccination.
    - Use proteomics-directed cloning to examine the dominant influenza specific clonotypes present in the serum prior to vaccination and at 14, 28, 90, and 365 days post-vaccination.
    - Use deep sequencing to track the frequency of each clonotype in the bone marrow plasma cell compartment at days 0, 28, and 365 post-vaccination and in the memory B-cell compartment at days 14, 28, 90, and 365 post-vaccination.
        - Identify which clones persist to day 365 post vaccination and examine whether the same plasmablast clones are re-amplified after each vaccination.
    - Identify consensus antibody sequences within the memory B-cell and long lived plasma cell compartments at day 28 and day 365 post vaccination for each clonotype.
        - Examine whether specific antibody features (hypermutation levels, antigen binding affinity, cross reactivity to previous vaccine strains) are associated with each compartment.
        - Examine whether these antibody features are correlated with the persistence of a given antibody family out to day 365.

4 STUDY DESIGN

Longitudinal assessment of the participants will be made at every study visit. Assessments will be performed at day 0, 7, 14, 28, 90 and 365 days after immunization as outlined in the following table. Participants will be screened for the presence of both expected reactions to vaccination and for unexpected adverse reactions and enrollment will take place within two weeks post-screening. Subject enrollment will commence with the availability of the seasonal flu vaccine and last for up to 365 days. See table in appendix A. Subjects will be offered the opportunity to participate in the study for up to 3 consecutive years provided eligibility criteria are met each year. Subjects who elect to continue in the study after the first year of participation will be rescreened to verify continued eligibility and re-consented prior to subsequent participation. Re-enrolling subjects will receive new subject identifiers and will count towards the total enrollment number for subsequent years of participation. Enrollment for the next year will begin with the availability of the seasonal flu vaccine. For subjects who elect to re-enroll in the study, the Day 365 visit (± 3 month window) would also be the Day 0 visit for the subsequent year.

Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study. Follow-up visits were also halted, which impacted the timing of participants' subsequent follow-up visits. For this study, there are participants whose Day 28 and Day 90 visits were impacted. We will request an expansion of the study window for the participants whose visits were impacted. Additional details regarding this are detailed further in the protocol. None of the other visits were impacted.

Dependent upon Emory's guidelines in response to the evolving nature of the pandemic and the prioritization of studies being allowed to re-open, time will be needed to secure the appropriate approvals and clearance to bring research participants back for their missed visits. As such, we would like to request that the missed visit windows for the Day 28 and Day 90 visits be extended for a maximum of up to 180 days, to ensure that ample time is available to bring participants back for their missed visits.
Enrollment for this study ended on March 31, 2020.

5 STUDY POPULATION

Up to 55 subjects will be enrolled from the existing population of healthy adults residing in the Atlanta, Georgia area within the Emory University, Children's Healthcare of Atlanta, and Centers for Disease Control Clifton Road community. We plan to enroll 15 subjects per year for 3 years and 10 subjects in year 4. Re-enrolling subjects in subsequent second and third years will count towards the yearly enrollment totals for those years.

Males and non-pregnant females aged 18 - 49 inclusive. Pregnancy status will be verified at screening, enrollment and prior to all bone marrow aspiration procedures. 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 word of mouth and through placement of flyers in key locations on campus. Social media messaging via Facebook and Twitter will also be used in an effort to increase the number of enrollees into this study. Based on previous experience, we anticipate enrollment of 40 to 50 percent non-pregnant females on the study.

5.1 Inclusion Criteria

For inclusion into the study, a subject must satisfy all of the following criteria:

1. Male or non-pregnant female subjects between 18 and 49 years of age (inclusive)
2. Subjects capable of providing written informed consent prior to initiation of any study procedures.
3. Subjects able to understand and comply with planned study procedures and be available for all study visits.
4. Safety labs
    a. WBC, within reference range of lower limit of normal of 4,000/ul, and upper limit of normal of 10,000/uL.
    b. Hemoglobin, within reference range of lower limit of normal of 11.4 gm/dL and upper limit of normal of 16.1 gm/dL.
    c. Hematocrit, within reference range of lower limit of normal of 33.3% and an upper limit of normal of 46.5%.
    d. Platelet Count within reference range of lower limit of normal of 150,000/uL and upper limit of normal of 400,000/uL.
    e. PT/INR, PT below or equal to the upper limit of normal of 13.1 seconds; INR within normal reference range of less than 1.5 (normal range for non-anti-coagulated patients).
    f. Creatinine within reference range of lower limit of normal of 0.4 mg/dL and upper limit of normal of 1.2 mg/dL.

g. Potassium), within reference range of lower limit of normal of 3.6 mM and upper limit of normal of 5.1 mM.

5. Heart rate ≥55 to ≤ 100 per minute
6. Systolic blood pressure ≥ 90 to ≤150 mm Hg
7. Diastolic blood pressure < 90 mm Hg
8. Oral temperature < 100ºF
9. Respirations even, unlabored, and > 10/minute to < 20/minute.
10. Female subjects of child bearing potential must have a negative urine pregnancy test at the screening visit, enrollment visit and prior to subsequent bone marrow aspirate procedures. Female subjects of childbearing potential must agree to practice abstinence, use a barrier method of birth control, or use an FDA approved form of birth control
11. Subjects who have not received seasonal flu vaccine for the current year (September – June)

Subjects that have participated in a previous year may be screened for re-enrollment in subsequent second and third year. Subjects that are re-enrolling will be re-consented, re-screened, and given a new identifier. Re-enrolling subjects will be counted towards the enrollment totals for each year. Subjects who report having already received the flu vaccine for the current flu season will be restricted from further study participation.

5.2 Exclusion Criteria

Subjects with any of the following will be excluded from the study:

1. If female, active pregnancy or breast-feeding or plans to become pregnant during study participation.
2. Subject report of having any medical disease or condition that, in the opinion of the site principal investigator or appropriate sub-investigator, is a contraindication to study participation. This includes any *acute or chronic*

medical disease or condition, defined as persisting 3 months (defined 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 or the subject's successful completion of this study.

3. Subject report of taking anticoagulants, or long-term (greater than 14 days) systemic steroids or other immunosuppressive medications
4. Subject report of known allergy to lidocaine.
5. Subject report of known hypersensitivity or allergy to eggs, egg or chicken protein, report of allergy to components of the study vaccine or other components of the study vaccine
6. Subject report of known latex allergy
7. Subject report of a history of severe reactions following previous immunization with licensed or unlicensed influenza virus vaccines
8. Subject report of a history of Guillain-Barre syndrome
9. Subjects who believe they cannot tolerate the bone marrow aspirations without sedation.
10. Subjects with an active infection or that have an acute illness within 72 hours prior to study vaccination. Subject having had an acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.
11. Subjects who are participating in another clinical trial involving the use of investigational agents or vaccines.

5.3 Handling of Withdrawals

Subjects are free to withdraw from the study at any time. Subjects are provided a revocation form at enrollment with withdrawal procedure clearly explained.

Subjects who do not meet inclusion and exclusion criteria at the second or third bone marrow aspirate visit will not have the aspiration performed and will be withdrawn from the study. Any unresolved adverse events will be followed per Section 9, Assessment of Safety.

Subjects may be taken off study without their consent if the study doctor determines that it is in the subject's best interest not to continue to participate in the study. In addition, the study doctor may remove a subject from study participation if the subject is unable to complete the required study procedures, or if the study is stopped by the institution, the sponsor, or the Food and Drug Administration (FDA) or other health authorities. If the subject is removed from the study, the Principal Investigator or designee will contact the participant to discuss the study stopping procedures.

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after study enrollment visit 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.

5.4 Termination of 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 monitoring review and recommendation, or at the discretion of DMID.

6 STUDY INTERVENTION PRODUCT

6.1 Study Product Description

Each flu season, the vaccine used in this study will be a licensed seasonal inactivated influenza vaccine for intramuscular injection that is approved by the FDA. The vaccine product used will depend upon supply stocked by the Emory University Hospital Investigational Drug Services (IDS).

6.1.1 Acquisition

The licensed vaccine will be provided by the Emory University Hospital Investigational Drug Pharmacy.

6.1.2 Formulation, Packaging, and Labeling

These vaccines are typically provided in pre-filled syringes (clear syringe plunger rod), 0.5 ml. If pre-filled syringes are not available from the manufacturer, the study product will be provided in a latex free single-dose vial, one vial per carton, 1 vial containing 0.5 ml dose.

The vaccine used in this study is the licensed seasonal inactivated influenza vaccine and will be supplied by Emory University Hospital Investigational Drug Services (IDS) in a pre-filled syringe or single-dose vial placed in a clear plastic bag labeled with the DMID protocol number, subject name, study ID, and dosing instructions per the prescription signed by the investigator's designee.

6.1.3 Product Storage and Stability

Per the package insert from the manufacturer, the seasonal inactivated influenza vaccine will be stored at 2-8 degrees C (35.6-46.4 degrees F). Vaccine will be stored at the Emory University Hospital Investigational Drug Services in a study specific designated location, within a temperature-controlled refrigerator. The temperature of the storage unit will be 24-hour, continuously recorded and monitored.

6.2 Dosage, Preparation and Administration of Study Intervention Product

A vaccine dose of 0.5 mL will be administered by the clinical research nurse intramuscularly (IM) in the subject's preferred deltoid (upper arm) per the manufacturer's instructions.

6.3 Modification of Study Intervention Product for a Participant

All subjects will receive 0.5 ml of vaccination. No modifications will occur for participants.

6.4 Accountability Procedures for the Study Intervention Product(s)

Emory Investigational Drug Service, as delegated by the study principal investigator, will maintain and monitor the inventory of the influenza vaccine per good clinical practice. Emory Investigational Drug Service will maintain records of study product acquisition, dispensing, storage and destruction.

6.5 Assessment of Participant Compliance with Study Intervention Product

Each participant will be assessed for compliance with the influenza vaccination via his or her receipt of 0.5ml of vaccine without immediate reaction.

6.6 Concomitant Medications/Treatments

Persons taking anticoagulants, long-term aspirin therapy, or long-term (greater than 14 days) systemic steroids will be excluded from study participation. Subjects with chronic medical conditions that require immunosuppressive medications which could alter immune function, or with chronic medical conditions such as diabetes or hypertension, will not be allowed to participate on the study. The medication history will be reviewed and updated at every study visit to ensure that subjects are not taking the medications prohibited for study participation.

Prohibited medications for this study include the following: tacrolimus, cyclosporine, mycophenolate mofetil, azathioprine, infliximab, enteracept, oral or intravenous steroids such as methylprednisolone, dexamethasone, prednisone; orencia, methotrexate, rapamycin, heparin, warfarin, low molecular weight heparins such as enoxaparin, and any medication not otherwise listed that, in the opinion of the investigator, meets the criterion as a prohibited medication for this study. The use of topical or inhaled steroids is allowed.

7 STUDY SCHEDULE

7.1 Screening

Pre-Vaccination, Screening: Day -14 to Day -1:

- When a potential participant is identified, participants will be provided with a description of the study (purpose and procedures) and asked to read and sign the informed consent. The informed consent will be signed prior to performing any study procedures, including any screening procedures. Demographic information will be collected from the participant.
- After providing written consent for the study, participants will receive a physical exam by a licensed to make medical diagnoses and listed on the Investigator of Record Form as the site principal investigator or sub-investigator. Subjects will have blood pressure, pulse rate, respiration rate and oral temperature recorded. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- They will have blood work drawn to determine if they are healthy enough for study participation as determined by careful assessment by the study physician as specified in Section 4. For these tests they will have about 2 teaspoons (10 cc) of blood drawn. Safety labs include WBC, Hemoglobin, Hematocrit, Platelet count, PT/INR Potassium and Creatinine. Subjects that would like re-enroll in the study for subsequent second and third years will have the same safety labs and must meet all eligibility criteria. Women of childbearing potential will have a urine pregnancy test and be included if the pregnancy test is negative. The lab test results must be assessed by the study physician using the most current available normal lab ranges and by determining the clinical significance for labs that fall outside of the normal range. The study physician will evaluate the safety labs and will determine if the subject is healthy enough to participate in the study.

- **Medical History:** Medical history will be obtained by interview of the subjects. Subjects will be queried regarding a history of significant medical disorders and a physical examination of the head, eyes, ears, nose throat, mouth, cardiovascular system, lungs, GI tract, nervous system, lymph glands, musculoskeletal system, and skin. will be performed. Medical histories of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse and autoimmune disease will be solicited by interview of the subjects. Subjects will be asked about any history of allergies.

- **Medications history:** All current medications, prescription and over-the-counter drugs taken in the past month (30 days) will be recorded. All vaccinations received within the past year will be recorded.

- Subjects with transient acute conditions (e.g., local infections, cold and/or flu symptoms, sprains, strains, and associated swelling and bruising) may be asked to return for screening after the transient condition resolves. Subjects

who do not meet inclusion criteria by the second visit will be ineligible for study enrollment.

7.2 Enrollment/Baseline

Day 0- Bone marrow aspiration, vaccination

- Study staff will reconfirm participants' willingness to take part in the study prior to performing any study procedures.
- Women of childbearing potential will have a urine pregnancy test.
- Subjects will have blood pressure, pulse rate, respiration rate and oral temperature recorded. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A peripheral blood draw of about 64 ml (4 tablespoon) of blood will be drawn to test baseline antibody levels.

Medical history will be reviewed and updated for any health changes since the screening visit as well as any updates to concomitant medications.

- Eligibility criteria, including results of all clinical screening laboratory evaluations and completion of the eligibility checklist, will be reviewed with participants and by the study staff to assure continued eligibility prior to study enrollment.
- Bone Marrow Aspiration: After having the bone aspirate marrow procedure explained to the subjects and signing the institutional consent for the procedure, the study physician will perform the bone marrow aspiration per institutional standard operating procedure.

Following bone marrow aspiration, the licensed seasonal trivalent inactivated influenza vaccine will be administered according to package insert by an intramuscular injection (shot) into the subject's preferred upper arm (deltoid muscle).

The Impact of COVID-19 on Study Visits:

- Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020. New enrollments were placed on hold for this study. Follow-up visits were also halted, which impacted participants' follow-up visits. Participant visits for Day 7 and Day 14 were not impacted.
  Follow-up visits for Day 28 and Day 90 visits were impacted.
- We are requesting that the windows for the Day 28 visit and the Day 90 visit be extended. Specific details are noted in the Day 28 and Day 90 follow-up visit sections below.
  Extending the windows for the Day 28 ad Day 90 follow-up visits would allow samples from participants whose visits were halted at these time points to be collected once the Emory restrictions regarding COVID-19 are lifted and staff can reasonably collect samples, dependent upon when the University and applicable Unit and School allow. The scientists feel that the samples, even if a few months late, will greatly help evaluate the longevity of the responses to flu vaccines.
- Study activities will not be changed, just the timing of the Day 28 and Day 90 visits. However, if a participant does not feel comfortable having a certain

study-related procedure done and declines a particular study-related procedure, they will have the option to do so.

- It is not anticipated that any of the Day 365 visits will be impacted.

Enrollment for this study ended on March 31, 2020.

7.3 Follow-up and Final Visits

- **Day 7 post vaccination study visit:** Subjects will have 64 ml of blood drawn for antibody testing. Vital signs, including oral temperature, blood pressure, pulse rate and respiratory rate will be obtained. A health assessment will be performed including an inquiry about any illness or health change since the last study visit as well as change in concomitant medication. Medical history will be reviewed and updated with any reported changes. An assessment will be made for any adverse events or serious adverse events. The Adverse Event Report Form will be updated if indicated.
- **Day 14 post vaccination study visit (+/- 2 days):** Subjects will have 64 ml of blood drawn for antibody testing. Vital signs including oral temperature, blood pressure, pulse rate and respiratory rate will be obtained. A health assessment will be performed including an inquiry about any illness or health change since the last study visit as well as change in concomitant medication. Medical history will be reviewed and updated with any reported changes. An assessment will be made for any adverse events or serious adverse events. The Adverse Event Report Form will be updated if indicated.

- **Day 28 post vaccination study visit (+/- 2 days):** Subjects will have clinical safety labs drawn including a platelet count and hemoglobin and hematocrit (4ml of blood) Females of childbearing potential will have a urine pregnancy test performed, and the test result must be negative for the subject to continue with study participation. The results of the blood tests must be assessed by the study physician as within a healthy range for continued study participation using the most current normal lab ranges, and evaluated for clinical significance of lab values that fall outside of normal range. Subjects will have 64 ml of blood drawn for antibody testing. Vital signs including oral temperature, blood pressure, pulse rate and respiratory rate will be obtained. A health assessment will be performed including an inquiry about any illness or health change since the last study visit. Concomitant medications will be reviewed and updated. An assessment will be made for any adverse events or serious adverse events. Participants will receive a physical exam by a licensed to make medical diagnoses and listed on the Investigator of Record Form as the site principal investigator or sub-investigator. The Adverse Event Report Form will be updated if indicated. Study eligibility criteria will be reviewed by the study nurse and physician and confirmed that the subject meets all inclusion and none of the exclusion eligibility criteria prior to the bone marrow aspirate procedure. Dr. Waller as site principal investigator or the sub-investigator will explain the bone marrow aspirate procedure, and the informed consent document for the procedure will be signed by the subject in the presence of Dr. Waller. The bone marrow aspirate procedure and aspirate volume will be the same as the same as at study enrollment.
    - Due to the COVID-19 pandemic, all non-essential research was halted in mid-March 2020.
    - One participant had their Day 28 visit halted.
    - We are requesting that the Day 28 window be extended by a maximum of up to 180 days, to allow completion of the follow-up visit for the impacted participant.
    - The participant will be contacted by phone or via encrypted email message to arrange scheduling of subsequent visits.
    - To assist with assessing symptoms, the COVID-19 Screening Questionnaire/Script will be reviewed with participants prior to bringing the participant in for follow-up visits.
- **Day 90 visit (acceptable range: days 80-120):** Subjects will have 64 ml of blood drawn for antibody testing. Vital signs including oral temperature, blood pressure, pulse rate and respiratory rate will be obtained. A health assessment will be performed including an inquiry about any illness or health change since the last study visit as well as change in concomitant medication. Medical history will be reviewed and updated with any reported changes. An assessment will be made for any adverse events or serious adverse events. The Adverse Event Report Form will be updated based on the subject's self-report.
    - Three participants had their Day 90 visit halted due to the COVID-19 pandemic.
    - We are requesting that the Day 90 window be extended by a maximum of up to 180 days, to allow completion of the follow-up visits for impacted participants.
    - Participants will be contacted by phone or via encrypted email message to arrange scheduling of subsequent visits.
    - To assist with assessing symptoms, the COVID-19 Screening Questionnaire/Script will be reviewed with participants prior to bringing them in for follow-up visits.

- **Day 365 visit (+/- 3 months):** Subjects will have clinical safety labs drawn including a platelet count and hemoglobin and hematocrit (4ml of blood). Females of childbearing potential will have a urine pregnancy test performed, and the test result must be negative for the subject to continue with study participation. The results of the blood tests must be assessed by the study physician to be within a healthy range for continued study participation using the most current normal lab ranges, and evaluated for clinical significance of lab values that fall outside of normal range. Subjects will have 64 ml of blood drawn for antibody testing. Vital signs including oral temperature, blood pressure, pulse rate, and respiratory rate will be obtained. A health assessment will be performed including an inquiry about any illness or health change since the last study visit. Concomitant medications will be reviewed and updated. An assessment will be made for any adverse events or serious adverse events. Participants will receive a physical exam by a licensed to make medical diagnoses and listed on the Investigator of Record Form as the site principal investigator or sub-investigator. The Adverse Event Report Form will be updated if indicated. Study eligibility criteria will be reviewed by the study nurse and physician and confirmed that the subject meets all inclusion and none of the exclusion eligibility

criteria prior to the bone marrow aspirate procedure. Dr. Waller will explain the bone marrow aspirate procedure, and the informed consent document for the procedure will be signed by the subject in the presence of Dr. Waller. The bone marrow aspirate procedure and aspirate volume will be the same as the as at study enrollment and at visit day 28.

- It is not anticipated that any of the Day 365 visits will be impacted.

The day 365 post vaccination visit will be the final study visit unless the subject decides to re-enroll in the study. Provided eligibility criteria are met, subjects may re-enroll in the study for a total study participation of up to three years per subject. The day 365 visit may also be the day 0 visit for a subject who is re-enrolling into the study, provided that re-screening of the subject occurs and the subject meets study eligibility criteria for continued study participation. These subjects will receive a new study identifier and a separate research record will be maintained by study staff.

7.4 Early Termination Visit

There are no special evaluations to be performed at a termination visit if early termination occurs, therefore there will be no visit for subjects who are terminated early from the study. The subject will be informed via telephone of the reason for early termination. The subject will not be brought back for any further visits. However, subjects that become pregnant during the study post enrollment will be followed by the team study team for safety monitoring monthly to the point of pregnancy outcome via telephone contact. Any adverse events that have not resolved at the early termination visit will be followed by phone contact for safety monitoring unless the principal investigator determines the adverse event is related to a study intervention and an unscheduled visit is necessary for safety monitoring.

7.5 Unscheduled Visit

Unscheduled visits are not anticipated with this study, but may occur at any time during the study. Any of the following activities may be performed:

- Medical history will be reviewed 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.
- Study personnel will discuss with participants and assess and record all AE/SAEs. Previously recorded AE/SAEs will be updated as appropriate.
- Vital signs, including oral temperature, pulse rate, blood pressure and respiration rate will be obtained. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Investigator of Record Form as the site principal investigator or sub-investigator.

- Approximately 10 mL of venous blood will be collected for chemistry and/or hematology performed by the local or site laboratory if deemed necessary by Dr. Waller or designee.

8 STUDY PROCEDURES AND EVALUATIONS

8.1 Clinical Evaluations

- Clinical interview of subject at screening to include:
  - Demographics: age, gender, ethnicity, race
  - History of allergies: food, drug, environmental, specifically solicit if any allergy to lidocaine or eggs
  - History of influenza vaccination, previous reaction
  - History of Guillain-Barre syndrome
  - History of medications taken within the past 30 days to include prescription and over the counter medications, specifically solicit if any blood thinners or immunosuppressive
  - History of any vaccinations within the past year
  - Acute or chronic medical conditions as reported by subject
- Vital signs at every study visit to include oral temperature, pulse rate, respiration rate, and blood pressure. Vital signs must be within acceptable limits: pulse rate ≥55 to ≤100 bpm; systolic BP between 90-150 mm Hg, oral temperature < 100°F, respirations even and unlabored ≥10/minute and < 20/minute.
- Physical exam at screening visit, Day 28 and Day 365 visits: subject height and weight per subject's self-report; clinical interview and physical examination by Dr. Waller with subject in the sitting and supine position where applicable to assess for any history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, GI tract, nervous system, lymph glands, musculoskeletal system, and skin. Physical exam of the body systems will be reported as normal, abnormal, and any abnormality will be described in detail.
- Collection of blood for clinical laboratory tests at screening visit, Day 28 and Day 365 study visits (10ml at screening, 4ml of blood at day 28 and day 365). Collection of research blood samples (64 ml per visit) will be collected at enrollment (Day 0), day 7, 14, 28, 90 and 365 study visits. Blood specimens will be drawn by trained phlebotomists at the Winship Cancer Institute. Urine pregnancy testing if subject is a woman of child-bearing potential will be performed by clinical research nurse at screening, enrollment (Day 0, Day 28 and Day 365 study visits. Clinical laboratory tests will be processed and reviewed by PI prior to aspiration procedures.
- Bone marrow aspirate procedure after eligibility verified with review of clinical laboratory test results and written consent obtained with the study physician at enrollment visit (Day 0), Day 28 and Day 365 visits: subject will be placed in the prone position on a gurney in one of the procedure rooms of the Winship Cancer Institute. The study physician will perform the procedure and be assisted by the clinical research nurse. The skin over the posterior iliac crest will be sterilely prepped and draped. Local anesthesia will be achieved by instillation of 2% lidocaine into the tissue surrounding the area where the

bone marrow aspirate will be removed. Using a sterile needle and using sterile technique about 30 cc of bone marrow will be aspirated via a heparinized syringe following a single puncture of the skin and periosteum. Following completion of removing up to 30 ml of bone marrow aspirate a sterile dressing will be applied. The bone marrow aspirate will then be transferred into four 10 ml sodium heparin tubes.

- Administration of licensed seasonal inactivated influenza vaccine by the clinical research nurse or designated staff at enrollment visit (Day 0) following the bone marrow aspirate procedure.
- Clinical health assessment at every study visit to include inquiry regarding any illness, any change in medication, and any overall health changes since last study visit.

8.2 Laboratory Evaluations/Assays

The participating laboratories are Emory Medical Laboratories (Emory University Hospital main laboratory).

Screening visit:
The following laboratory evaluations are included in the screening visit:

- Hematology: Hemoglobin, hematocrit, white blood cells (WBC), platelet count.
- Biochemistry: potassium, creatinine
- PT/INR
- Urine pregnancy testing for women of childbearing potential

Day 0:
The following laboratory evaluations are included in the enrollment visit (Day 0):

- Urine pregnancy test for women of childbearing potential prior to the bone marrow aspirate procedure
- Research Serology for immune response (64 ml of blood)
- Repeat of any screening labs if it is determined that there has been a health change that may indicate a change in one of the screening labs.
- Research sampling of bone marrow aspirates (30ml of marrow)

Day 7, 14, 90:
The following laboratory evaluations are included at day 7, 14, and 90 post vaccination

- Research Serology for immune response (64 ml of blood)

Day 28, 365:
The following laboratory evaluations are included on visit day 28 and 365 post vaccination

- Urine pregnancy test for women of childbearing potential prior to the bone marrow aspirate procedure
- Research Serology for immune response (64 ml of blood)
- Hematology: hemoglobin, hematocrit, platelet count.
- Research sampling of bone marrow aspirates (30ml of marrow)

Laboratory Assays:

- Longitudinal tracking of vaccine-induced B cell responses with special emphasis on broadly neutralizing HA stem reactive responses: ASC responses in blood and bone

marrow will be tracked by ELISPOT, which provides a quantitative measurement of ASC numbers in which each cell creates a spot on a plate based on the adherence of the antibodies which it produces to a capture antigen. Peripheral blood mononuclear cells or bone marrow mononuclear cells will be isolated using CPT tubes or histopaque purification, respectively. cells will be seeded into ELISPOT wells coated with reagents for capturing total IgG, IgM, or IgA (to measure total ASC numbers), or coated with the influenza vaccine (to measure total vaccine-specific responses), or coated with the head or stem domains of the influenza HA antigen (to measure the specific HA domains targeted by the response). To measure antigen-specific bone marrow plasma cell responses, which are low in frequency, ASCs will be enriched using magnetic bead separation prior to coating in ELISPOT plates.

- Clonotypic analysis of influenza specific memory B-cells and plasma cells in the blood and bone marrow of vaccinated subjects. Responding ASCs present in the blood at day 7 post vaccination will be sorted by flow cytometry and seeded into PCR plates for amplification of Ig heavy and light chain genes. Related antibody rearrangements present in multiple wells will be cloned and expressed as recombinant proteins to determine their reactivity towards influenza. The frequency of these clones in the bone marrow ASC compartment as well as the blood ASC and memory B-cell compartments will be determined using bulk RNA isolated from sorted bone marrow plasma cells. We determine the frequency of each clone over time in this bulk RNA using both Next Generation Sequencing (NGS) analysis and quantitative PCR using clonotype-specific primers and Taqman fluorescent probes.
- Defining the antibody-intrinsic features that promote long-term persistence of bone marrow plasma cells and memory B-cells. The sequences of vaccine-specific clonotypes in the bone marrow at early and late time points following vaccination will be determined from the NGS data. We will analyze these sequences for their level of somatic hypermutation, using tools available on the International Immunogenetics information system (IMGT) website. We will determine the impact of these mutations on affinity for antigen by expressing mutated or unmutated versions of the parental clone as recombinant proteins followed by ELISA and Biacore assays.
- Using proteomics-directed cloning to examine influenza specific clonotypes in the long-lived plasma cell compartment. In collaboration with Cell Signaling Technology we will be able to examine influenza specific plasma cell responses which contribute significantly to the serum response but may not be part of the blood ASC response at day 7 post vaccination. To do this, we will enrich influenza specific antibodies from donor plasma on an antigen-coated column, followed by protein sequencing of these antibodies and comparison to the NGS libraries generated from bulk bone marrow ASC RNA from the same donor. This approach allows us to identify influenza specific responses that are present at baseline prior to immunization and represent long-lived cells.

8.2.1 Specimen Collection, Preparation, Handling and Shipping

Samples will be drawn, labeled, and logged at Emory's Winship Cancer Institute by a skilled clinical research nurse or phlebotomist, under the supervision of Dr. Edmund Waller. Blood samples will be drawn and stored in 8ml CPT tubes. Bone marrow aspirate specimens will be collected in 10 ml sodium heparin tubes and labeled with coded subject information. They will

be maintained at room temperature until they are transported to Dr. Ahmed's laboratory. Samples distributed to the Ahmed laboratory will only be identifiable by an assigned donor number, day of vaccination, study identifier and a draw date. Samples will be transported in sealed biohazard containers between sites per standard protocol. PBMC and serum samples will be used fresh or will be banked for future batch analyses. All blood and PBMC samples will be handled in biosafety hoods with the handling personnel wearing protective safely gear (lab coat, gloves, and safety glasses when required). Samples will be transported in sealed biohazard containers from Dr. Waller's laboratory to Dr. Ahmed's laboratory per standard protocol.

Subjects consented to participate in this study will also be asked to consent to storage of any unused blood and bone marrow specimens for future assays. These samples will not be used for genetic (DNA) studies. De-identified frozen PBMC and bone marrow samples will be stored in liquid nitrogen at the Emory Vaccine center and/or at Rollins Research Center. Serum samples will be stored in -80 °C ± 10 ° C freezers at the Emory Vaccine center and/or at Rollins Research Center.

8.3 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Any laboratory values falling outside of the protocol defined range of normal will be evaluated by RN and PI for clinical significance. For subjects with laboratory values in the clinically significant range, the lab abnormalities will be explained to the study participant by Dr. Waller or a designee. Also, the subject will be given a copy of his or her lab report and instructed to share and follow up with his or her primary health care provider or health clinic. The study team will follow AEs until resolution.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Only FDA approved, licensed seasonal trivalent inactivated influenza vaccine will be administered in this protocol as standard of care during influenza vaccine season, following the manufacturers' instructions and safety precautions.

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

9.2.1 Adverse Events

Adverse Event:

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or stabilization. In the judgment of the PI, any unexpected reaction to the vaccine will be reported to VAERS with a copy faxed to CROMS Pharmacovigilance.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it will be recorded as an AE.

All AEs will be graded for severity and relationship to study product.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system: mild, moderate, and severe as defined below:

- Mild: events require minimal or no treatment and do not interfere with the subject's daily activities.

- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent will require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician will assess an AE's relationship to influenza vaccine as part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs will have their relationship to study product assessed using the terms: related or not related using the following guidelines:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

For the purposes of this study, reportable adverse events are limited to any occurrence or worsening of an undesirable or unintended sign, symptom or disease that is specifically associated with a study procedure that is not part of the normal standard of care for the participant. As previously mentioned, 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, subjects may experience "vaso-vagal syndrome" during phlebotomy. Vaso-vagal reactions may include diaphoresis, nausea, syncope and rarely loss of consciousness. Severe vaso-vagal reaction, including loss of consciousness or > grade 2 hypotension will be considered reportable adverse events.

Anticipated adverse events related to administration of the inactivated influenza vaccine include: localized erythema, induration, swelling, itching and/or pain at the injection site, mild fever, myalgia, headache and malaise following vaccination. Since immunization with flu vaccine annually is offered as standard of care to healthy adults to prevent influenza infection, these anticipated reactions to the vaccine will not be considered reportable adverse events. Illness requiring hospitalization as deemed related to vaccination will be reported as an adverse event.

This study also involves the aspiration of bone marrow which involves some risk to the subject. The physical risks of undergoing a bone marrow aspiration are pain and bruising that may last 1 to 3 days. Very rarely more serious side effects could occur including damage to normal blood vessels, veins or bone structures, as well as an infection in the tissue surrounding the aspirate site or in the bone itself. The removal of 30 cc of marrow produces a temporary and mild anemia

that may last one or two weeks. This anemia should be well tolerated by healthy subjects who have been screened prior to participating in the vaccination study.

Adverse events will be followed until resolved or considered stable.

Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine will be considered an adverse event for the study.

Per the VAERS Table of Reportable Events Following Vaccination found using the following link http://vaers.hhs.gov/resources/VAERS Table of Reportable Events Following Vaccination.pdf, any adverse event described in the manufacturer's insert as a contraindication to additional doses of vaccine that occur within the time frame per the package insert for the specific licensed vaccine administered in this study or in the judgment of the PI, any unexpected reaction to the vaccine will be reported to VAERS with a copy faxed or emailed to the CROMS Pharmacovigilance Group.

All adverse events that meet the reporting requirements of the Institutional Review Board will also be faxed or emailed to the Clinical Project Manager. Per Emory Institutional Review Board reporting guidelines, these adverse events would include those that are unanticipated, related to the study intervention and involve risk to the study participant.

9.2.2 Serious Adverse Events

Possible serious adverse events from venipuncture are a severe vaso-vagal reaction, including loss of consciousness or ≥ grade 2 hypotension will be considered reportable adverse events. A possible serious adverse event would an illness deemed related to influenza vaccination requiring hospitalization. Very rare more serious adverse events from the bone marrow aspirate procedure would include damage to normal blood vessels, veins or bone structures, as well as an infection in the tissue surrounding the aspirate site or in the bone itself.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

Serious Adverse Event (SAE): Serious Adverse Events will be collected from the time the participant begins the study until study completion, or until the participant withdraws from the study. SAE's will be documented on the Adverse Event form and reported to the DMID Pharmacovigilance Group.

All SAEs, regardless of relationship will be recorded on the SAE form and sent by fax to CROMS Pharmacovigilance within 24 hours of site awareness of the event.

A Serious Adverse Event is defined as an AE meeting one of the following conditions:

- Death during the period of protocol defined surveillance

- Life Threatening Event (defined as a subject 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 subject 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.

Events **not** considered to be serious adverse events are hospitalizations for the:

- routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen
- treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission

Any AE that meets a protocol-defined serious criterion will be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

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

9.3.2 Other Adverse Events (if applicable)

Not applicable

9.3.3 Reporting of Pregnancy

A negative urine pregnancy testing is required for all women of childbearing potential prior to the bone marrow aspirate procedure at day 0, day 28, and day 365. If the pregnancy test is positive the subject will be excluded from any further participation in the study. No further study procedures will be conducted in the event of a positive pregnancy test and subject will not return to site for any follow up visits. Subjects that become pregnant during the study post enrollment will be followed by the team study team for safety monitoring monthly to the point of pregnancy outcome via telephone contact.

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

Clinical studies at Emory University are compliant with ICH E6 guidelines for Good Clinical Practice.

Adverse events will be documented and reported according to the DMID and Emory IRB policies (as described in section 9.2.1 and 9.3). All AEs and SAEs will be followed until resolved, return to baseline or stable as determined by the study team. The occurrence of AEs and the status of existing AEs will be assessed at each study visit and events are recorded in the regulatory binder. Subjects will be encouraged to contact study staff of any health changes that may occur between study visits. An unscheduled visit or telephone follow-up may occur depending on the adverse event.

9.5 Halting Rules

The study will be halted if the following occurs:

- One or more participants have laryngospasm, bronchospasm, or anaphylaxis associated with study procedure and occurring within 72 hours post study procedure;
- Any SAE associated with study product or procedure;
- Two or more participants experience the same or similar severe or life-threatening AE judged to be associated with study procedure.

9.6 Safety Oversight

Safety oversight is the responsibility of the Independent Safety Monitor (ISM). The ISM will be approved by DMID in accordance with DMID guidelines. Serious and severe adverse events will be reported to the ISM and DMID. The DMID Medical Monitor has the authority to stop study enrollment, vaccinations and procedures if AEs are reported that meet halting criteria.

9.6.1 Independent Safety Monitor (ISM)

An ISM at the site will review adverse events in a timely fashion and ensure that appropriate management is initiated and completed at the site. The ISM will have direct contact with the Principal Investigator and follow all events on an on-going basis. Any issues or questions will be directed to the DMID CPM.

10 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.

10.1 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. Emory University IRB and other regulatory agencies may conduct study monitoring visits.

11 STATISTICAL CONSIDERATIONS

For this study we will assess immunologic responses in the periphery (blood) and centrally (bone marrow). These responses contain a variety of immunologic assays with different types of response output. Hence our primary data analytic strategy will be the use of longitudinal data analysis methods to determine the temporal course of a given response measurement and factors associated with heterogeneity in that temporal course. Study outcomes will be measured using numerical data from laboratory assays including flow cytometry and ELISPOT assays. A detailed data management system will utilize general linear mixed models for continuous responses to assess differences among time points.

11.1 Study Hypothesis

To investigate the longevity of humoral immunity to influenza virus in humans. Our overarching hypothesis is that an understanding of how long-term humoral immunity to influenza virus is generated and maintained is essential for the development of a “universal” vaccine against influenza virus. This aim will be accomplished by measuring the total influenza-specific and hemagglutinin stem-specific bone marrow plasma cells, memory B-cells, and serum antibody levels over extended periods following vaccination.

11.2 Sample Size Considerations

A major goal our study is to understand the longevity of plasma cell responses directed against different determinants in the influenza vaccine. Our preliminary data has suggested that plasma cell clonal responses against conserved epitopes may be smaller in number and less prone to persist compared to responses against epitopes which are unique to the particular hemagglutinin strains used in the current year's vaccine. Our deep sequencing data suggests that after one year the frequency of the average vaccine-induced clone in the bone marrow is approximately 33% of the frequency observed at one month. However, there is a large variability in this maintenance (standard deviation of 44%). The small number of cross-reactive clones we have tracked so far have been undetectable after one year, suggesting that their maintenance is substantially lower than the average clone. We wish to be 80% confident in being able to detect whether the maintenance of these cross-reactive clones is at least 3-fold lower than for the average clone (i.e. 11% maintenance vs. 33%) with a P-value less than 0.05. This will require the longitudinal tracking of at least 25 broadly cross reactive plasma cell clones in our patient cohort. Our initial data suggests that we should be able to identify a single broadly cross-reactive clonotype in approximately 50% of our donors, meaning that a sample size of 50 donors will be required to follow 25 cross-reactive clonotypes. With our planned sample size of 55 donors, this allows for a potential dropout rate of 10%.

11.3 Planned Interim Analyses (if applicable)

Not Applicable

11.3.1 Safety Review

Not Applicable

11.3.2 Immunogenicity or Efficacy Review

Not Applicable

11.4 Final Analysis Plan

We will use the unpaired Student's t-test to compare the frequencies of strain-specific and broadly cross-reactive clonotypes in the marrow after one year as expressed as a percentage of their frequencies at one month post vaccination. We will also work to develop a multi-phase exponential decay model for plasma cell persistence in the blood and bone marrow over time. We will use ANOVA to evaluate the contributions of antibody specificity (head vs. stem specific; broadly reactive versus strain specific reactivity), affinity for antigen, and level of hypermutation on the observed exponential decay constants of individual clones in each decline phase that we identify through our modeling. This work will be performed in collaboration with the Emory/UGA CEIRS Data Management Team headed by Dr. Hertzberg using the approved Data Management Plan for this contract.

12 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. Laboratory reports will serve as source documents for lab privi required at screening and prior to bone marrow aspirate procedures. All study documents will be secured by key and/or password protection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

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

The Principal Investigator will provide direct access to source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

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

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 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).

14.2 Institutional Review Board

The Emory University IRB will review and provide approval of this protocol and the associated informed consent documents, recruitment materials and procedures as required by OHRP and local requirements before subject enrollment. The Emory University IRB currently holds and will maintain a US FWA issued by OHRP for the entirety of the study. Any amendments to the protocol or consent materials will be approved before they are placed in use. An independent ethics committee may review the study at any time.

14.3 Informed Consent Process

Informed consent will be 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 subjects. Consent forms describe in detail the study procedures and risks, and written documentation of informed consent is required prior to enrollment in the study. Consent forms are IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the research nurse will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. They will have ample opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects 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, or withdraw from, this study.

An additional "Consent to Surgical or Medical Treatment" is required by Emory Healthcare (attached) prior to the bone marrow aspirate procedure on day 0, 28 and day 365.

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

Not applicable

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

Not applicable

14.5 Participant Confidentiality

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 Emory IRB may also have access to the subject's record.

To protect privacy, the NIH has provided a Certificate of Confidentiality, CoC. With this CoC, the participating sites cannot be forced to release information that may identify the participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The participating sites will use the CoC to resist any demands for information that would identify the participant, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study or local laws, such as for reporting of communicable diseases.

A CoC does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive study information, then the participating sites may not use the CoC to withhold that information.

The CoC does not prevent the participating sites from reporting without the participant's consent, information that would identify the subject as a participant in the study regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14. 6 Protocol Deviations:

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice (GCP), or protocol-specific Manual of Procedures requirements or institution SOPs. The noncompliance may be either on the part of the subject, 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 PI/study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 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, will be addressed in study subject source documents. The original document of the DMID Protocol Deviation (PD) Form will be maintained in the Regulatory File, as well as a copy kept in the subject's source document file. Protocol deviations will be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14.7 Study Discontinuation

If the study is discontinued, participants will be contacted via telephone and in writing. Due to the nature of this study, it is not anticipated that an enrolled study participant will require continued follow-up after study closure.

14.8 Future Use of Stored Specimens

Participants who agree to take part in this study will have specimens collected for antibody determination and viral typing. Each specimen will be labeled only with a unique tracking number to protect participant's confidentiality. Participants will be asked, as part of the informed consent process, to agree to or refuse his/her specimen to be used only for future research as noted in the protocol and consent form. This decision can be changed at any time by the participant without penalty.

Residual plasma, bone marrow cells, and peripheral blood mononuclear cells, will be used for measuring additional immune correlates of the long term antibody response to influenza

vaccination. These studies will include 1) correlating antibody response to T-cell responses after influenza vaccination; 2) profiling RNA expression and immunoglobulin specifies of subsets of memory B cells and plasma cells in the blood and bone marrow; 3) determining whether the generation of certain memory B cell phenotypes is associated with the development of long lived bone marrow plasma cells; and 4) determining antibody titers to hemaglutinin proteins contained in the vaccine. Some of these studies may require generation of monoclonal antibodies from sorted antibody-secreting cells and cloning of influenza- specific immunoglobulin genes from vaccinated subjects.

15 DATA HANDLING AND RECORD KEEPING

The 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.

15.1 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.

15.2 Data Capture Methods

Both paper and electronic recording of data will be used in this study. Paper data will be scanned and electronically uploaded to a central clinical database (RedCap) that is password-protected and HIPAA compliant.

Exposure data will be captured on CRFs after interviewing the participants. Laboratory data will be recorded on CRFs upon completion of the laboratory analysis. CRFs may be used as source documents if they represent data collected for the study and are where data were initially recorded. All data will be entered into one clinical database, RedCap.

If data are obtained at a later date (i.e., after the study visit) and are recorded on the CRF as source documentation, it will be signed/initialed and dated.

If data are transcribed from another source onto the CRF, the CRF is not considered to be the original source document and it will not be used as source documentation.
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.

15.3 Types of Data

Data for this study will include safety assessments and research laboratory assays data.

15.4 Timing/Reports

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

15.5 Study Records Retention

Records and documents pertaining to the conduct of this clinical study, including CRFs, source documents, and consent forms will 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 will be retained for a longer period, however, if required by local regulations.

16 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.

17 LITERATURE REFERENCES

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(6) Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity.* 1998 Mar;8(3):363-72.

18 SUPPLEMENT/APPENDICES

Appendix A, Schedule of Events

Analysis of Bone Marrow and Blood B cell Immune Responses to Influenza Vaccination

Procedures	**Baseline/Screening (Day-14-Day-1)**	Day 0	7d	14d	28d	90d	365d
Study Consent	X						
Medical History	X						X
Physical Exam including Vital Signs	X				X		X
Baseline Labs	X				X**		X**
Health Assessment, AE/SAE	X	X	X	X	X	X	X
Pregnancy Testing	X*	X*			X*		X*
Enrollment		X					X***
Consent for Bone Marrow aspirate		X			X		X
Bone Marrow Aspirate Procedures/Samples		X			X		X
Peripheral blood draw for research immunity testing		X	X	X	X	X	X
Administration of Influenza Vaccine		X					X***
Re-enrollment (optional)							X

* for women of child-bearing potential prior to any invasive procedures

** Platelet Count and HGB/HCT

*** For subjects enrolling in subsequent second and third years, Day 365 will include a review of eligibility and administration of vaccine.

Appendix B, Study Flow Chart