TITLE: ASSESSMENT OF THE EFFECT OF AGE AND PRIMING ON THE IMMUNOLOGICAL RESPONSE TO AN INACTIVATED VACCINE FOR NOVEL H1N1 VIRUS.

Protocol Number: URMC 09-005

Principal Investigator:

John Treanor, MD

Version Number: 1.0

Date 30 SEP 2009

STATEMENT OF COMPLIANCE

This clinical trial will be conducted in accordance with the protocol and Good Clinical Practices (GCP) as required by the following:

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

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

SIGNATURE PAGE

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

Principal Investigator:

Signed: _____ Date: _____

Name: John Treanor, MD

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

A/Cal/07/2009	Influenza A/California/07/2009 (H1N1)
AE	Adverse Event/Adverse Experience
BARDA	Biomedical Advanced Research and Development Authority
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
eCRF	Electronic CRF
FDA	Food and Drug Administration
GMT	Geometric Mean Titer
GCP	Good Clinical Practice
HA	Hemagglutinin
HAI	Hemagglutination-Inhibition Assay
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IM	Intramuscular
IND	Investigational New Drug application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
mcg	Micrograms
MedDRA®	Medical Dictionary for Regulatory Activities
MN	Microneutralization
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NA	Neuraminidase
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health, DHHS
N1	NA of the N1 subtype
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PHI	Protected Health Information

PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TIV	Trivalent inactivated influenza vaccine
US	United States
USP	United States Pharmacopoeia
WHO	World Health Organization

PROTOCOL SUMMARY

Title: ASSESSMENT OF THE EFFECT OF AGE AND PRIMING ON THE IMMUNOLOGICAL RESPONSE TO AN INACTIVATED VACCINE FOR NOVEL H1N1 VIRUS.

Phase: IV

Population: A total of 60 healthy adult subjects who do not have a prior history of novel H1N1 influenza infection or vaccination will be enrolled in three age groups, 18 to 32 years, 60 to 70 years, and over 70 years, and receive a single dose of licensed monovalent H1N1 influenza vaccine in open label fashion..

Number of Sites: Single site

Duration of study 5 months

Duration of subject participation: 2 months

Description of Agent or Intervention: Licensed egg-derived monovalent inactivated novel H1N1 influenza vaccine intramuscularly .

Primary objectives:

The primary objective of this study is to determine the relationship between prevaccination baseline immune status and the immune response to a single dose of inactivated H1N1 influenza vaccine.

Secondary objectives:

Secondary objectives include:

- Description of the magnitude and phenotype of the B cell response to vaccination
- Description of the T cell specificity before and after vaccination
- Evaluation of the safety of vaccination

Primary endpoints:

The primary endpoint for determination of the immune response to vaccination will be the development of serum antibody assessed by hemagglutination-inhibition (HAI) and neutralization tests.

The primary endpoint for assessment of the safety of vaccination will be the rate and severity of solicited adverse events within seven days of vaccine.

Secondary endpoints:

Secondary endpoints will include:

- The number (assessed by ELISPOT analysis) and functionality (assessed by antibody assay of culture supernates) of the B cell response to vaccination
- The number of antigen specific T cells and the specific recognition of influenza peptides
- The frequency and magnitude of hemagglutinin-specific mucosal IgA response assessed by ELISA on nasal secretions.

Study Design

The study will be conducted as an open-label, prospective evaluation in healthy adults in three age groups representing a spectrum of prior exposure to H1N1 viruses. Because the study site has already experienced significant novel H1N1 activity, young adult subjects will be screened for detectable serum antibody to novel H1. In addition, elderly subjects with a known history of prior vaccination with A/New Jersey/76 (H1N1) vaccine will be excluded. Eligible subjects will receive a single dose of licensed inactivated A/California/07/09 (nH1N1) vaccine.

Baseline priming will be assessed by (1) age, reflective of previous exposure to H1N1 viruses, and (2) baseline memory B cell and CD4 T cell reactivity to the A/California//07/09 HA. The subsequent response to vaccination will be assessed by measurement of nasal and serum antibody, and peripheral blood B cell and T cell responses at day 7, 14, and 28 after vaccination. Safety of vaccination will be assessed using symptoms collected for 7 days after vaccine.

1 KEY ROLES

Principal Investigator:

John J Treanor, MD
Department of Internal Medicine
Division of Infectious Diseases, Room 3-6308
University of Rochester Medical Center
601 Elmwood Avenue
Rochester, NY 14642
Tel: 585-275-5871
Fax: 5855-473-9863
Email: John_Treanor@urmc.rochester.edu

Scientific Investigators:

Kanta Subbarao, M.D.
Brian Murphy, M.D.
Catherine Luke, Ph.D.
Laboratory of Infectious Diseases, NIAID, NIH
Building 33, Room 3E13C.1
33 North Drive, MSC 3203, Bethesda, MD 20892
301-451 3839 (phone)
Email: ksubbarao@niaid.nih.gov

RCHSPB Regulatory Affairs:

John Tierney, R.N.
RCHSPB/NIH/NIAID
6700-B Rockledge Dr. Room# 1112
MSC 7609, Bethesda, MD 20892
301-451-5136 (phone)
Email: jtierney@mail.nih.gov

Independent Safety Monitor

Michael Keefer, MD
Department of Internal Medicine
Division of Infectious Diseases, Room 3-6305
University of Rochester Medical Center
601 Elmwood Avenue
Rochester NY 14642

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Human H1N1 infections:

Human infections with novel H1N1 viruses of swine origin were first identified in April of 2009. The prototype virus of this outbreak, A/California/04/2009 virus, is a so-called quadruple reassortant, deriving the PA and PB2 gene segments from avian influenza A viruses of North American lineage, the PB2 gene segment from a human H3N2 virus, the HA, NP, and NS gene segments from a swine virus of North American lineage, and the NA and M gene segments from a swine virus of Eurasian lineage [1].

The H1 HA of the novel H1 virus is an example of a classical swine H1 HA, and is highly divergent from the HA of seasonal H1 influenza A viruses. There is an approximately 25% difference between the HAs of A/California/04/09 and the seasonal H1 of A/Brisbane/08 virus on an amino acid level, with the changes mostly concentrated in known antibody binding epitopes in the globular head of the HA1 component. Antigenically, the A/California/04/09 HA is most closely related to the HA of the swine A/New Jersey/76 virus and to H1 influenza viruses circulating early after the introduction of H1 viruses in humans in 1918. There is little or no cross reactivity between the novel and seasonal H1 HAs using hemagglutination-inhibition (HAI) assays, and unexposed persons less than 60 years of age do not have detectable antibody against the A/California/04/09 virus. Neither seasonal live nor inactivated influenza vaccines induce immune responses that can recognize or would be expected to provide significant protection against the novel H1 virus [2].

Triple reassortant swine influenza viruses which do not contain gene segments from Eurasian swine viruses have been detected in swine in North America since 1998. These viruses have caused sporadic human infections, some of which have been severe, but have not resulted in sustained human-to-human transmission [3, 4]. The acquisition of the Eurasian swine NA and M gene segments appears to have conferred on the novel H1 virus a much greater degree of transmissibility, easily demonstrated in ferret and guinea pig animal models, and clearly shown by the rapid world-wide spread of these viruses among humans. Thus these H1N1 viruses have both the quality of antigenic novelty and the quality of efficient transmission required for a new pandemic influenza virus. In the less than 50-day period between their first identification on April 24, 2009 and June 11, 2009 global spread of the novel H1N1 viruses had reached the criteria for stage 6 of the WHO pandemic stages, and the outbreak was officially declared a pandemic.

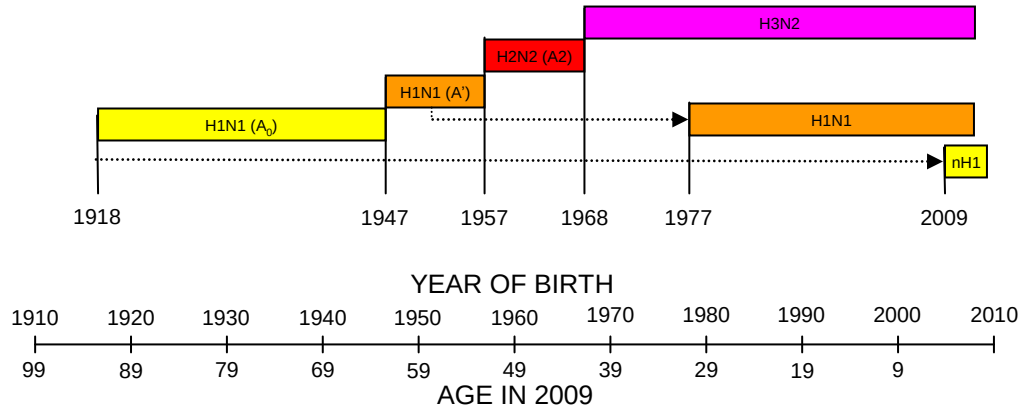
Information regarding the clinical characteristics of illness due to novel H1N1 viruses is still being gathered, but preliminary observations have suggested that these viruses cause typical

influenza, possibly with an increased frequency of gastrointestinal complaints compared to typical seasonal disease. The severity of the disease appears to be roughly similar as well, but because of the extraordinarily high attack rates, the total burden on the health care system has been overwhelming in some localities, and there are many cases requiring hospitalization and intensive care unit care. Current isolates are generally resistant to adamantanes and sensitive to neuraminidase inhibitors, but there have been recent reports of detection of oseltamivir-resistant viruses including at least one from an individual with no documented exposure to oseltamivir.

Rapid deployment of effective vaccines against novel H1N1 viruses will be a critical component of measures to control the pandemic and mitigate its impact. Currently, 4 vaccines are licensed in the US based as a "strain change" based on manufacturing data: inactivated influenza vaccines formulated at 15 ug of HA antigen by CSL limited, sanofi, and Novartis, and live, cold-adapted vaccine containing 10^7 $TCID_{50}$ per dose manufactured by MedImmune. Surprisingly, early clinical data has suggested that healthy adults between 18 and 64 years of age respond strongly to a single dose of 15 ug of subvirion inactivated vaccine [5]. Similar data from other studies of inactivated vaccines have been reported in press releases, including the announcement by NIAID that 96% of healthy adults developed serum HAI antibody titers of 40 or greater within 14 days of receiving a single dose of inactivated vaccine. Based on the labeling for seasonal vaccine, it is expected that both inactivated and live vaccines will be recommended as a single dose in individuals 9 years of age and above, and as a two dose schedule in children under 9 with no history of previous influenza vaccination.

2.2 Rationale

Although the original origins of H1N1 viruses are not completely clear, historical evidence currently supports the hypothesis that these viruses were simultaneously introduced into swine and human populations from an unknown animal (possibly avian) reservoir at some point shortly before the Spanish Flu pandemic of 1918. Since that time, there has been significant antigenic evolution of H1N1 viruses in humans under the selective pressure of population immunity, while there has been substantially less evolution in H1 viruses among swine. Thus, the current A/California/07/09 virus is antigenically most closely related to the H1N1 viruses that circulated in humans in 1918, and human isolates since 1918 are progressively more distinct. This observation would predict that age, reflecting the possibility of exposure to antigenically related H1N1 viruses, would be an important factor determining both potential resistance to infection, as well as priming for responses to vaccination, as illustrated in the figure below. Epidemiologic studies of the novel H1 pandemic to this point have revealed a striking predilection for younger individuals with relatively little disease occurring in individuals 60 and older [6], consistent with the possibility that exposure to more closely related H1N1 viruses, particularly before 1947, results in some level of residual protective immunity. However, the response to a single dose of vaccine clearly does not depend on exposure to these older H1 influenza viruses.



Recent chronology of influenza A viruses in humans: The pandemic virus of 1918 was an H1N1 virus (influenza A_0) closely related to viruses that became established in swine ("classical swine influenza"). In 1947 a significant antigenic change in H1 viruses was observed (distinct enough to be referred to as influenza A' in the literature of that time). A' viruses circulated until 1957 when they were replaced by H2N2 viruses (influenza A2). In 1968 H3N2 viruses emerged and replaced H2N2 viruses, and have continued to circulate in humans until now. In 1977, H1N1 viruses re-emerged which antigenically resembled H1N1 viruses circulating in humans in 1950. The current novel H1 viruses in humans are antigenically related to classical swine H1 viruses.

Thus, other priming mechanisms independent of age must also be involved in the vigorous responses of adults to inactivated novel H1N1 influenza vaccines [5]. At this point, the data are most consistent with the interpretation that previous exposure to seasonal H1N1 viruses are sufficient to prime for responses to inactivated novel H1N1 viruses. The specific immunological mechanisms responsible for priming are unknown, but could involve cross-reactive memory B cells, cross reactive CD4 T-cell epitopes on the HA, or both.

In order to assess the role of baseline cross reactive CD4 cells and memory B cells on the response to live and inactivated influenza vaccines, we will recruit healthy adults who have not been previously exposed to novel H1N1 viruses, and vaccinate them with a single dose of licensed inactivated novel H1N1 vaccine. We will assess age, and baseline numbers of antigen-specific B cells and CD4 cells as indications of previous priming, and determine the CD4 and CD8 response and epitope specificity, memory B cells, antibody secreting cells (ASC), and serum and mucosal anti-influenza antibody on days 7, 14, 28, and 56 following vaccination as indicators of the effect or priming on the kinetics and magnitude of the subsequent response.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

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

In placebo-controlled trials in adults, inactivated seasonal vaccines are associated with mild local pain at the site of administration. Systemic symptoms like fever and malaise occur at rates equal to placebo. During the swine influenza vaccine campaign of 1976, about 10 per 1,000,000 vaccine recipients in excess of the background rate developed the paralytic illness called Guillain-Barré Syndrome (GBS). In the subsequent decade, no association between seasonal influenza vaccine and GBS was found. More extensive investigations of this potential association occurring in the 1990s revealed that there was a small but detectable risk of GBS in the 6 weeks following seasonal influenza immunization: an attributable risk of approximately 1 per 1,000,000, adjusted for potential confounders. In the period since the Vaccine Adverse Event Reporting System (VAERS) was established in 1990, the rates of GBS reports following influenza vaccination have declined substantially. The annual reporting rate in that period was highest in the 1993-1994 influenza season (1.7 per 1,000,000 vaccinees) and lowest in the last season analyzed in the report, 2002-2003 (0.4 per 1,000,000 vaccinees) [7]. No cases of GBS have been reported following receipt of novel H1N1 influenza vaccines to date, but the total number of recipients is only in the thousands. Most persons who develop GBS recover completely.

Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur but can be prevented or mitigated by applying direct pressure to the draw site for several minutes. The use of alcohol swabbing and sterile equipment will make infection less likely at the site where blood will be drawn. Risks occasionally associated with nasal wash include pain or discomfort, and very rarely, epistaxis.

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

2.3.2 Known Potential Benefits

It is likely that vaccination with the novel H1vaccine will result in an immune response that could provide protection against illness due to novel H1N1 viruses. The duration of any such immune response is currently unknown. Receipt of the novel H1N1 vaccines is unlikely to afford any substantial protection against seasonal influenza viruses.

3 OBJECTIVES AND ENDPOINTS

3.1 Primary objectives:

The primary objective of this study is to determine the relationship between prevaccination baseline immune status and the immune response to a single dose of inactivated H1N1 vaccine.

3.2 Secondary objectives:

Secondary objectives include:

- Description of the magnitude and phenotype of the B cell response to vaccination
- Description of the T cell specificity before and after vaccination
- Evaluation of the safety of vaccination

3.3 Primary endpoints:

The primary endpoint for determination of the immune response to vaccination will be the development of serum antibody assessed by hemagglutination-inhibition and neutralization tests.

The primary endpoint for assessment of the safety of vaccination will be the rate and severity of solicited adverse events within seven days of vaccine.

3.4 Secondary endpoints:

Secondary endpoints will include:

- The number (assessed by ELISPOT analysis) and functionality (assessed by antibody assay of culture supernates) of the B cell response to vaccination
- The number of antigen specific T cells and the specific recognition of influenza peptides
- The frequency and magnitude of hemagglutinin-specific mucosal IgA response assessed by ELISA on nasal secretions.

4 STUDY DESIGN

The study will be conducted as an open-label, prospective evaluation in healthy adults in three age groups representing a spectrum of prior exposure to H1N1 viruses. Because the study site has already experienced significant novel H1N1 activity, young adult subjects will be screened for detectable serum antibody to novel H1. In addition, elderly subjects with a known history of prior vaccination with A/New Jersey/76 vaccine will be excluded. Eligible subjects will receive a single dose of licensed inactivated A/California/07/09 (nH1N1) vaccine.

Baseline priming will be assessed by (1) age, reflective of previous exposure to H1N1 viruses, and (2) baseline memory B cell and CD4 T cell reactivity to the A/California/09 HA. The subsequent response to vaccination will be assessed by measurement of nasal and serum antibody, and peripheral blood B cell and T cell responses at day 7, 14, and 28 after vaccination. Safety of vaccination will be assessed using symptoms collected for 7 days after vaccine.

5 STUDY POPULATION

5.1 Subject Inclusion Criteria

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

1. Aged between 18 and 32 years inclusive, or 60 years or older.

2. No prior history of laboratory documented infection with novel H1N1 virus or immunization with novel H1N1 vaccine.

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

4. Female subjects of childbearing potential must have a negative pregnancy test (urine or serum) within 24 hours prior each to vaccination.

5. The subject must be in good health, as determined by: vital signs (heart rate <100 bpm; blood pressure: systolic ≥ 90 mm Hg and ≤140 mm Hg; diastolic ≤ 90 mm Hg; oral temperature <100.0ºF); medical history; and targeted physical examination, when

necessary, based on medical history. Stable medical condition is defined as: no recent increase in prescription medication, dose, or frequency of medication in the last 3 months and health outcomes of the specific disease are considered to be within acceptable limits in the last 6 months.

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

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

5.2 Subject Exclusion Criteria

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

1. Subjects with a previous history of vaccination against novel H1N1 virus or a laboratory documented history of previous novel H1N1 infection.

2. Subject has history of previous vaccination with A/New Jersey/76 vaccine (for subjects 60 and older).

3. Subject has a history of egg allergy or is allergic to other components of the vaccine.

4. The subject is a woman who is pregnant or breastfeeding or intends to become pregnant during the study period between enrollment and 30 days following receipt of vaccine.

5. The subject is immunosuppressed as a result of an underlying illness or treatment with immunosuppressive or cytotoxic drugs, or use of anticancer chemotherapy or radiation therapy within the preceding 36 months.

6. The subject has an active neoplastic disease (excluding non-melanoma skin cancer or prostate cancer that is stable in the absence of therapy) or a history of any hematological malignancy. For this criterion, “active” is defined as having received treatment within the past 5 years.

7. The subject has long-term (greater than 2 weeks) use of oral or parenteral steroids, or high-dose inhaled steroids (>800 mcg/day of beclomethasone dipropionate or equivalent) within the preceding 6 months (nasal and topical steroids are allowed).

8. The subject received immunoglobulin or another blood product within the 3 months prior to enrollment in this study.

9. The subject has received an inactivated vaccine within the 2 weeks or a live vaccine within the 4 weeks prior to enrollment in this study or plans to receive another vaccine within the next 28 days.

10. The subject has an acute or chronic medical condition that, in the opinion of the investigator, would render vaccination unsafe or would interfere with the evaluation of responses. These conditions include chronic conditions recognized as risk factors for influenza complications or as contraindications for live vaccination, including chronic cardiac (exclusive of hypertension) or pulmonary conditions (including asthma), diabetes mellitus, or renal impairment.

11. The subject has an acute illness or an oral temperature greater than 99.9°F (37.7°C) within 3 days prior to enrollment or vaccination. Subjects who had an acute illness that was treated symptoms resolved are eligible to enroll as long as treatment is completed and symptoms resolved > 3 days prior to enrollment.

12. The subject is currently participating or plans to participate in a study that involves an experimental agent (vaccine, drug, biologic, device, blood product, or medication) or has received an experimental agent within 1 month prior to enrollment in this study, or expects to receive another experimental agent during participation in this study, or intends to donate blood during the study period.

13. The subject has any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

14. The subject has a diagnosis of schizophrenia, bi-polar disease, or other severe (disabling) chronic psychiatric diagnosis, or are receiving psychiatric drugs. Subjects who are receiving a single antidepressant drug and are stable for at least 3 months prior to enrollment without decompensation are allowed enrollment into the study.

15. The subject has a history of alcohol or drug abuse in the 5 years prior to enrollment.

16. The subject has a known human immunodeficiency virus, hepatitis B, or hepatitis C infection.

17. The subject has a previous history of Guillain-Barré syndrome within 6 weeks of receipt of influenza vaccine.

18. The subject has any condition that the prinicipal investigator (PI) believes may interfere with successful completion of the study.

5.3 Modification and Discontinuation of Study Intervention/Investigational Product for a Subject

5.3.1 Dose/Schedule Modifications for a Subject

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

5.3.2 Criteria for Discontinuation of Study Intervention/Product for Withdrawal of a Subject

Follow-up of subjects will be discontinued if any of the following criteria are met:

- The PI decides that it is in the best interest of the subject or the study for the subject to discontinue participation. For example, issues of noncompliance with visits may prompt discontinuation.
- The subject withdraws consent. Subjects may withdraw their consent for study participation at any time during the study without penalty.
- The subject is lost to follow-up.
- The study is terminated.

6 STUDY INVESTIGATIONAL PRODUCT

6.1 Study Product Acquisition

6.1.1 Formulation, Packaging and Labeling

Inactivated vaccine will consist of the licensed, egg-derived inactivated subvirion A/California/07/09 monovalent vaccine, formulated at a dose of 15 mcg of HA antigen per 0.5 mL dose as assessed by single radial immunodiffusion (SRID) or FDA-approved equivalent metholodogy and supplied in single dose syringes. Inactivated vaccine will be manufactured by Novartis and supplied by the Biomedical Advanced Research and Development Agency (BARDA), US Department of Health and Human Services.

6.2 Product Storage and Stability

Influenza vaccines will be stored in secure, limited-access temperature monitored refrigerator environment at 2°C to 8°C (35.6°F to 46.4°F) until needed. DO NOT FREEZE. The

temperature of the storage unit will be monitored during the duration of the trial, and documentation of proper dedicated storage will be maintained. In the event of accidental deep-freezing or disruption of the cold chain, vaccines will not be administered; and the PI or the responsible person will contact the sponsor for further instructions.

6.3 Preparation, Administration, and Dose of Study Intervention/Investigational Product

Vaccineswill be provided in unit dose syringes and will not require additional formulation prior to administration. Inactivated vaccine will be administered at a dose of 15 mcg HA antigen by deep intramuscular injection in the deltoid muscle.

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

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

6.5 Concomitant Medications/Treatments

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

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

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

7 STUDY PROCEDURES/EVALUATIONS

7.1 Enrollment/Randomization/BLINDING Procedures

This is an open-label, non randomized study.

7.2 Clinical Evaluations

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

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

Targeted Physical Examination: Licensed study clinicians (i.e., physician, physician's assistant, nurse practitioner) may conduct a targeted physical examination, if necessary to assist in determining eligibility. All subjects will have vital signs (blood pressure, pulse, and oral temperature) measured prior to vaccination.

Reactogenicity Assessments: Study personnel will take a brief history of subjects for assessment of AEs. A targeted physical examination, which may include an assessment of erythema, induration, pain, and tenderness at the injection site, may be performed at follow-up visits after vaccination.

Memory Aids: Study personnel will review the subject memory aids to elicit reactogenicity data and AE information.

7.3 Laboratory Evaluations

7.3.1 Clinical Laboratory Evaluations

Urine or serum pregnancy tests will be performed within 24 hours prior to vaccination on all female subjects of childbearing potential. No other screening or clinical laboratory evaluations will be routinely performed.

7.3.2 Immunogenicity Evaluations

Serum will be obtained prior to, and on days 7, 14, and 28 following vaccination. Serum antibody will be evaluated by:

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

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

Nasal secretions will be obtained by nasal wash on days 0 and 28 and will be tested for antibody by kinetic ELISA. Secretions will be concentrated 10-fold and tested by solid phase ELISA using baculovirus-expressed A/California/07/09 hemagglutinin antigen, and antibody detected using monoclonal antibody specific for human secretory IgA. Titers will be corrected for total IgA content.

B cell responses: Peripheral Blood Mononuclear Cells (PBMC) will be obtained before and on day 7 and 28 after vaccine and evaluated for B-cell responses. B cell responses will be evaluated by memory cell assay [8] and antibody secreting cell assay. In addition, B cells will be cultured in vitro and the functional quality of secreted antibody assessed by HAI and MN assays

CD4 and CD8 T cell responses PBMC collected prior to and on day 14 after vaccination will be evaluated for the presence of antigen specific CD4 cells by gamma-interferon ELISPOT assay using a panel of peptides spanning the nH1 hemagglutinin [9]. This assay will identify both cross reactive and nH1 specific epitope responses. PBMC will also be evaluated for the presence of antigen specific ELISPOT and cell homing markers by flow cytometry.

HLA typing: In order to aid in the assessment of cellular responses, subjects will have complete HLA genotyping by high-throughput PCR amplification and genomic sequencing analysis

7.3.3 Specimen Preparation, Handling, and Shipping

7.3.3.1 Instructions for Specimen Preparation, Handling, and Storage

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

7.3.3.2 Specimen Shipment

Instructions for specimen shipment are included in the MOP.

8 STUDY SCHEDULE

8.1 Screening and Enrollment

8.1.1 Screening Visit (day -7 to day -28)

Subjects expressing interest in the study will be invited to the study site for a screening visit. At the screening visit, the study, and its risks and benefits will be explained to the subject, a brief medical history will be obtained, and a sample of peripheral blood obtained for assessment of serum antibody to novel H1N1 virus in subjects under 32 years of age.

8.1.2 Enrollment/Vaccination Visit (visit 1, day 1)

Subjects who qualify by history and (for 18-32 year olds) who have levels of serum HAI antibody ≤ 1:8 will be enrolled in the study and receive the first dose of vaccine.

A complete medical history will be obtained with particular attention to inclusion and exclusion criteria, and a directed physical exam performed as indicated by medical history. Females of childbearing potential will have a urine pregnancy test which must be negative prior to vaccination. Inclusion and exclusion criteria will be reviewed using a checklist and the eligibility of the subject for study participation will be verified.

A sample of 10 mL of peripheral blood will be obtained in a serum-separator tube for assessment of serum antibody, and an additional 50 mL of blood obtained in a heparinized tube for preservation of peripheral blood mononuclear cells (PBMCs). These latter cells will be preserved in liquid nitrogen for later studies.

Nasal wash for assessment of mucosal immunity will be obtained by lavage of the nasopharynx with 10 mL of sterile lactated ringers solution.

Subjects will receive licensed subvirion inactivated novel H1N1 vaccine by deep intramuscular injection in deltoid of the non dominant arm.

Following vaccination, subjects will remain in the vaccine clinic for a minimum of 30 minutes prior to discharge to observe for any immediate vaccine adverse events. Prior to discharge, subjects will be given a memory aid on which to record local and systemic symptoms, and a thermometer for recording oral temperature daily.

8.2 Subject Follow-Up

Subjects will return for follow-up evaluations on days 7, 14, and 28 after vaccination for assessment of symptoms and cellular and humoral immune responses.

8.2.1 Visit 2, day 7 (Window days 6-8)

At this visit, the memory aid will be reviewed with the subject, and any significant new symptoms evaluated. 10 mL of sera will be obtained in a serum separator tube and 50 mL of blood will be obtained in heparinized tubes for collection and preservation of PBMC.

8.2.2 Visit 3, day 14 (Window days 13-15)

At this visit, the subject will be queried for new adverse events, and 10 mL of sera will be obtained in a serum separator tube and 50 mL of blood will be obtained in heparinized tubes for collection and preservation of PBMC.

8.2.3 Visit 4, day 28 (Window days 27-29)

At this visit, the subject will be queried for new adverse events. 10 mL of blood will be obtained in a serum-separator tube and 50 mL of blood in heparinized tubes for assessment of serum antibody and cellular responses to vaccination. In addition, nasal wash will be obtained for assessment of nasal antibody to influenza.

8.3 Early Termination Visit

If subjects discontinue from the study, they will be asked to make an early termination visit. At the time of the early termination visit, the reason for early termination will be recorded, current health status since the last visit will be reviewed, and all concomitant medications will be recorded. A targeted physical examination may be performed, as indicated, and information regarding AEs will be solicited. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established.

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

8.4 Illness visits

Subjects reporting influenza-like illness (fever or feverishness plus either cough or sore throat on the same or consecutive days) during the period from 7 to 28 days after vaccination will return for an illness visit within 72 hours of symptom onset. At the illness visit, medical history will be obtained, targeted physical exam will be performed, and nasopharyngeal swab obtained for PCR evaluation for respiratory viruses.

8.5 Unscheduled visits

Any unscheduled visits will be documented, including the reason for the visit, documentation of the findings and any actions taken.

9 ASSESSMENT OF SCIENTIFIC OBJECTIVES

9.1 Specification of the Appropriate Outcome Measures

Primary and secondary outcome measures are described in the protocol (refer to Section 3).

9.2 Methods and Timing for Assessing, Recording, and Analyzing Appropriate Outcome Measures

The schedule for collecting safety, reactogenicity, and blood samples for serum and cellular assays (immunogenicity data) are found in the study table in the appendix.

Information concerning specimen collection, processing and shipping is contained in the MOP.

10 ASSESSMENT OF SAFETY

10.1 Adverse Events, Reactogenicity, Serious Adverse Events

10.1.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in an investigation subject administered the vaccine and does not necessarily have a causal relationship with the vaccination. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational vaccine whether or not related to it. Exacerbation of pre-existing conditions and

intercurrent illnesses will be recorded as AEs. Stable chronic conditions which are present prior to enrollment and do not worsen are not considered AEs and will be accounted for in the subject's medical history. All AEs must be graded for relationship to the investigational vaccine and severity.

10.1.2 Reactogenicity

Reactogenicity events (REs) are predefined AEs that can potentially occur after vaccine administration. All REs will be assessed on the Isolation Unit during the acute phase of this study. The following will be considered REs:

Event	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	LIFE-THREATENING
Chills	Chills causing no or minimal interference with usual social & functional activities	Chills causing greater than minimal interference with usual social & functional activities & requiring non-narcotic medication	Chills causing inability to perform usual social & functional activities	N/A
Conjunctivitis	Conjunctivitis causing no or minimal interference with usual social & functional activities	Conjunctivitis causing greater than minimal interference with usual social & functional activities	Conjunctivitis causing inability to perform usual social & functional activities	N/A
Cough	No interference with activity, may use cough drops	Some interference with activity or use of non-narcotic medication	Significant, prevents daily activity and requiring use of narcotic medication	Emergency Room (ER) visit or hospitalization
Epistaxis	Epistaxis causing no or minimal interference with usual social & functional activities	Epistaxis causing greater than minimal interference with usual social & functional activities	Epistaxis causing inability to perform usual social & functional activities and requiring medical intervention (e.g., nasal packing)	Epistaxis requiring hospitalization or blood transfusion
Headache	No interference with activity; may require use of non-narcotic pain reliever	Some interference with activity or use of non-narcotic pain reliever $\geq$3 times per episode	Significant, prevents daily activity and requiring use of narcotic pain reliever	ER visit or hospitalization

Event	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	LIFE-THREATENING
Wheezing/Rhonchi	Sustained wheezing, rhonchi, or crackles on physical examination without dyspnea	Sustained wheezing, rhonchi, or crackles on physical examination with dyspnea	Sustained wheezing, rhonchi, or crackles on physical examination with dyspnea AND need for supplemental oxygen	Respiratory failure with ventilator support indicated
Myalgia	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities & requiring non-narcotic medication	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions & requiring ER visit or hospitalization
Otitis media	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Pharyngitis	Pharyngitis causing no or minimal interference with usual social & functional activities; may require use of non-narcotic pain reliever or lozenges	Pharyngitis causing greater than minimal interference with usual social & functional activities or requiring use of non-narcotic pain reliever $\geq$3 times or any use of antibiotics	Pharyngitis causing inability to perform usual social & functional activities and requiring use of narcotic pain reliever and/or outpatient IV hydration	Threatens airway integrity and/or requires hospitalization
Rhinorrhea/Nasal congestion	Rhinorrhea or nasal congestion causing no or minimal interference with usual social & functional activities	Rhinorrhea or nasal congestion causing greater than minimal interference with usual social & functional activities	N/A	N/A

All AEs other than fever and reactogenicity events will be assessed by the study investigators using the following grading system:

Table 4: Adverse Event Grading System

Severity	Defined
Grade 0 (None)	N/A

Table 4: Adverse Event Grading System

Severity	Defined
Grade 1 (Mild)	No effect on activities of daily living, over the counter treatment given ≤2x/day
Grade 2 (Moderate)	Partial limitation in activities of daily living (can complete ≥50% baseline, or treatment given >2x/ day)
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline. Medical intervention often required.
Grade 4 (Life-threatening)	Inability to perform basic self-care functions OR severity of illness requires hospitalization

Severity of fever will be assessed using the following grading system:

Table 5: Fever Grading System

Reactogenicity Event	Grade	Intensity
Fever (oral) If temperature is ≥100.4°F, will be confirmed by repeating temperature after waiting 20 minutes	0	<100.4°F (<38.0°C)
	1	≥100.4°F – 101.4°F (≥38.0°C – 38.6°C)
	2	≥101.5°F – 102.4°F (38.7°C – 39.1°C)
	3	>102.4°F (>39.1°C)

10.1.3 Serious Adverse Event

A serious adverse event (SAE) is an AE that is determined to be “serious” based on subject/event outcome or action criteria, whether considered related to the investigational vaccine or not. An SAE results in one of the following outcomes:

1. Death during the period of protocol-defined surveillance.

2. Life threatening event: defined as an event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe.
3. Hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance: defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting.
4. Congenital anomaly or birth defect.
5. Persistent or significant disability or incapacity; defined as a substantial disruption of the study subject's ability to carry out normal life functions.
6. Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon the judgment of the PI, it may jeopardize the health of the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed above.

Each AE will be classified by the PI as "serious" or "nonserious". An AE needs to meet only 1 of the above criteria to be considered serious. A change in vital signs or laboratory test results will be considered to be an SAE if the change continues to meet one of the above criteria upon urgent retesting (no later than 48 hours).

10.2 Reporting Procedures

10.2.1 Serious Adverse Event Detection and Reporting

Adverse events including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected for unsolicited adverse events includes event description, date of onset, investigator assessment of severity, investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome. The intensity and causality of non-serious AEs will be assessed by a licensed clinician (i.e., medical doctor, nurse practitioner, physicians assistant). All AEs occurring during the AE reporting period of the study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or until considered stable.

Any medical condition that is present at screening will be considered a baseline condition and will not be reported as an AE. If the severity of any pre-existing medical condition increases during the study period, then it will be recorded as an AE.

All SAEs will be:

- Assessed for intensity and causality by a licensed clinician.
- Recorded on the appropriate SAE report form.
- Followed through resolution by a study physician.

- Reviewed by an Independent Safety Monitor, the sponsor, and the Institutional Review Board (IRB).

Any AE considered serious by the PI or Subinvestigator or that meets the aforementioned criteria must be submitted on an SAE form to the sponsor at the following address:

Kanta Subbarao, M.D.
Laboratory of Infectious Diseases, NIAID, NIH
Building 33, Room 3E13C.1
33 North Drive, MSC 3203, Bethesda, MD 20892

Timelines for submission of an SAE form are as follows:

- All deaths and life-threatening events regardless of relationship will be recorded on the SAE form and sent by fax within 24 hours of site awareness of the death or life-threatening event.

- All other SAEs, regardless of relationship, will be reported via fax by the site within 72 hours of becoming aware of the event.

- Other supporting documentation of the event may be requested by the pharmacovigilance contractor and should be provided as soon as possible.

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

All SAEs will be reviewed by a study physician, recorded on the appropriate SAE Reporting Form, and followed through to resolution by a study physician. All SAEs will be reported by telephone, email, or fax within 1 working day of notification of the SAE occurrence to all of the following:

- Dr. Kanta Subbarao, NIAID/NIH, (301) 451-3839
- Dr. Michael Keefer, Medical Monitor, Phone: (585) 275-5871

Following notification from the PI, Dr. Subbarao, as the representative of the Sponsor, will report events that are both serious and unexpected and that are unlikely, possibly, probably, or definitely related to the vaccine, to the FDA via MedWatch forms..

10.2.2 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported. All study-mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Pregnancies will be followed to pregnancy outcome pending the subject's permission.

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

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

10.2.4 Type and Duration of the Follow-up of Subjects After Adverse Events

Adverse events will be followed until resolved or considered stable.

10.3 Safety Oversight (ISM)

Safety oversight will be under the direction of the PI and an Independent Safety Monitor (ISM). Serious and severe adverse events will be reported to the sponsor, and the ISM..

10.3.1 Safety Monitoring Committee (SMC)

Because this study evaluates a licensed product, there will not be a formal safety monitoring committee

10.3.2 Independent Safety Monitor (ISM)

The ISM will review serious 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 ongoing basis.

10.4 Halting Rules

There are no formal halting rules for this study.

11 DATA HANDLING AND RECORD KEEPING

11.1 Source Documents and Access to Source Data/Documents

Complete source documentation (laboratory test reports and hospital or medical records) is required for every study subject for the entire duration of the study. Data from source documentation for subjects enrolled in the study will be entered into an electronic medical record system that will be compatible with the Clinical Research Information Management System of the NIAID (CRIMSON) Data System. The data entry is to be completed on an ongoing basis during the study. Data entry shall be performed by authorized individuals. Corrections to the data system shall be tracked electronically (password protected) with time, date, individual making the correction, and what was changed. Source documentation should support the data entered**,** and must be signed and dated by the person recording and/or reviewing the data.

11.2 Data Management Responsibilities

The PI is responsible for the accuracy, completeness, and timeliness of the data reported to the Sponsor. All data entered should be reviewed by an Investigator and signed as required with written or electronic signature, as appropriate. Data reported should be consistent with source documents or the discrepancies should be explained. Source documentation will be made available for review or audit by the Sponsor or designee and any applicable Federal authorities

11.3 Data Capture Methods

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

11.4 Types of Data

Data for this study will include safety, laboratory, and outcome measures (e.g., reactogenicity, virology, and immunogenicity).

11.5 Study Records Retention

The PI is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. All essential documentation for all study subjects are to be maintained by

the investigators in a secure storage facility for a minimum of 3 years, in accordance with the NIH FWA. No study document should be destroyed without prior written agreement between RCHSPB/NIAID and the PI. Should the PI wish to assign the study records to another party and/or move them to another location, the PI must provide written notification of such intent to RCHSPB/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written NIAID permission must be received by the site prior to destruction or relocation of research records.

11.6 Protocol Deviations

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

These practices are consistent with ***Good Clinical Practice: Consolidated Guideline***: Section 4, **Investigator;** Section 5, **Sponsor,** Section 6, **Clinical Trial Protocol and Protocol Amendments**.

It is the responsibility of the site 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. All deviations must be promptly reported to the sponsor.

All deviations from the Protocol must be addressed in study subject source documents. A completed copy of the Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff are responsible for knowing and adhering to their IRB/IEC requirements.

11.7 Site Monitoring Plan

The Sponsor will monitor all aspects of the study with respect to current Good Clinical Practices and for compliance with applicable government regulations. Prior to the start of the study, the PI will be informed of the frequency of monitoring visits and will be given reasonable notifications prior to each visit. The objectives of a monitoring visit will be to verify the prompt reporting of SAEs, to verify the physical presence of the signed Informed Consent forms, and to compare CRFs or CRIMSON data extractions and spreadsheets with source data for completeness and accuracy. During the monitoring visit, the PI (and/or designee) and other study personnel should be available to discuss the study. Study documents must be available for review throughout the course of the study.

12 STATISTICAL CONSIDERATIONS

12.1 Introduction

This study is exploratory rather than confirmatory; its purpose is to estimate event rates and patterns of immune responses rather than to test formal statistical hypotheses. Sample size was determined based on prior similar Phase I vaccine trials. Descriptive approaches will be used as well as formal statistical tests as outlined below. Results will be presented in tabular format, as well as graphically, where appropriate

12.2 Study Objectives

Primary objectives:

The primary objective of this study is to determine the relationship between prevaccination baseline immune status and the immune response to a single dose of inactivated H1N1 vaccine.

Secondary objectives:

Secondary objectives include:

- Description of the magnitude and phenotype of the B cell response to vaccination
- Description of the T cell specificity before and after vaccination
- Evaluation of the safety of vaccination

12.3 Study Outcome Measures

The primary endpoint for determination of the immune response to vaccination will be the development of serum antibody assessed by hemagglutination-inhibition and neutralization tests.

The primary endpoint for assessment of the safety of vaccination will be the rate and severity of solicited adverse events within seven days of vaccine.

Secondary endpoints will include:

- The number (assessed by ELISPOT analysis) and functionality (assessed by antibody assay of culture supernates) of the B cell response to vaccination
- The number of antigen specific T cells and the specific recognition of influenza peptides

- The frequency and magnitude of hemagglutinin-specific mucosal IgA response assessed by ELISA on nasal secretions.

12.4 Sample Size Considerations

Given a sample size of 20 subjects in each group, the half width of the confidence interval for any dichotomous outcome is shown in the table below:

P	half width
5%	10%
10%	13%
15%	16%
20%	18%
25%	19%
30%	20%
35%	21%
40%	21%
45%	22%
50%	22%

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written site quality management plan, the investigational site is responsible for conducting routine quality assurance (QA and quality control (QC) activities to internally monitor study progress and protocol compliance. The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the sponsor.

The Data Manager chosen for this trial will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The PI 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 (IRB)

The institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate. Refer to: http://ohrp.osophs.dhhs.gov.

Prior to enrollment of subjects into this trial, the approved protocol and the informed consent form will be reviewed and approved by the appropriate IRB and submitted to the FDA. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB and submitted to the sponsor.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to the sponsor. Notification of the IRB's composition, or the IRB's Federal-wide Assurance number, will be provided.

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

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this intervention will be provided to the subjects. Consent forms will describe in detail the study interventions, products, study procedures, and risks to the subject and written documentation of informed consent is required prior to starting intervention or administering study product. Consent forms will be approved by the IRB and the subject will be asked to read and review the document. Upon reviewing the document, the PI will explain the research study to the subject and answer any questions that may arise. The subjects should have the opportunity to discuss the study with their family and/or friends and think about

it prior to agreeing to participate. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects may withdraw consent at any time throughout the course of the trial. 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 this study.

The PI will choose subjects in accordance with the eligibility criteria detailed in section 5. The investigator will not exercise selectivity so that bias is prevented. All subjects will sign an informed consent form that complies with the requirements of both 21 CFR Part 50 and Health Insurance Portability and Accountability Act (HIPAA) before entering the trial. Or, a consent form that complies with the requirements of 21 CFR Part 50 and a separate HIPAA compliant authorization form for the use and disclosure of the subject's protected health information may be used instead, per institutional standard operating procedures.

Prior to participation in the trial, subjects will receive a comprehensive explanation of the proposed vaccine research, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their blood samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them. The consent form will not include any exculpatory statements.

The sponsor will provide the PI, in writing, any new information that significantly bears on the subjects' risk to receive the investigational product. This new information will be communicated by the PI to subjects who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be asked to again provide consent, if necessary, due to changes.

Site staff may employ screening procedures with a screening consent and script approved by their IRB. However, a signed informed consent is required prior to conducting any protocol-specific procedures. Subjects will be given a copy of all consent forms that they sign.

By signing the informed consent form, the subject agrees to complete all evaluations required by the trial, unless the subject withdraws or is terminated from the trial for any reason.

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

Pregnant women are excluded from participation in this study because blood drawing volumes are limited by CFR in pregnant women for studies without direct benefit. This study will include both adults of both genders and there are no exclusions based on race.

14.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor(s), and their agents. This confidentiality extends to biological sample tests, in addition to the clinical information relating to participating subjects.

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 PI. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. Clinical study sites will permit access to such records.

14.6 Study Discontinuation

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

14.7 Compensation

Subjects will be compensated for participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

14.8 Future Use of Stored Specimens

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

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

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

15 PUBLICATION POLICY

Following completion of the study, the PI may publish the results of this research in a scientific journal. The trial will be registered in ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM), in accordance with the NLM requirements under the FDAAA.

16 LITERATURE REFERENCES

1. team Nii. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. New England Journal of Medicine 2009;360:2605-2615

2. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. New England Journal of Medicine 2009;361:epub ahead of print

3. Newman AP, Reisdorf E, Beinemann J, et al. Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. Emerging Infectious Diseases 2008;14:1470-1478

4. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States 2005-2009. New England Journal of Medicine 2009;360:2616-2625

5. Greenberg ME, Lai MH, Hartel GF, et al. Response after one dose of monovalent 2009 influenza A (H1N1) vaccine - preliminary report. New England Journal of Medicine 2009;361:epub ahead of print

6. CDC. Update: Novel influenza A (H1N1) infections - Mexico, March - May 2009. Morbitity Mortality Weekly Report 2009;58:585-589

7. Haber P, DeStefano F, Angulo FJ, et al. Guilllain-Barre syndrome following influenza vaccination. JAMA 2004;292:2478-2481

8. Crotty S, Aubert RD, Glidewell J and Ahmed R. Tracking human antigen-specific memory B cells: a sensitive and generalized ELISPOT system. Journal of Immunological Methods 2004;286:111-122

9. Sant AJ, Chaves FA, Krafcik FR, et al. Immunodominance in CD4 T-cell responses: implications for immune responses to influenza virus and for vaccine design. Expert Review of Vaccines 2007;6:357-368

17 SUPPLEMENTS/TABLE OF PROCEDURES

Study day ->	D-7 to -28	0	7	14	28		Blood
Procedure	Screen	V1	V2	V3	V4		Volume
Informed Consent	X						
Medical History	X						
Screening serum antibody*	X						
Interval History		X					
Directed Physical Exam		X					
Urine Pregnancy Test**		X					
Review Entry Criteria		X					
Serum for antibody (10 mL)		X	X	X	X		40
Nasal wash antibody		X			X		
PBMC (50 mL)		X	X	X	X		200
Vaccination		X					
Symptom memory aid		X	X				
Collect memory aid			X				
* - subjects 18 to 32 only ** - Female subjects of childbearing potential only							