A Controlled Human Infection Study of Influenza A/Bethesda/MM2/H1N1 Virus (A/California/04/2009/H1N1-like) in Healthy Subjects to Assess the Effect of Pre-Existing Immunity on Symptomatic Influenza Virus Infection

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH): Good Clinical Practice (GCP) E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry," published in the Federal Register (83 Federal Register 8882 (2018)
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this 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 E6 GCP guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the Sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Name

Title

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LIST OF ABBREVIATIONS

°C	Degree Celsius
°F	Degree Fahrenheit
ADL	Activities of Daily Living
AE	Adverse Event/Adverse Experience
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
ASC	Antibody Secreting Cells
AST	Aspartate Transaminase
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHI	Controlled Human Infection
CK	Creatinine Kinase
Cl	Chloride
cMRI	Cardiac MRI
CMS	Clinical Material Services
CO_2	Carbon Dioxide
CoP	Correlation of Protection
COPD	Chronic Obstructive Pulmonary Disease
Cr	Creatinine
CRF	Case Report Form
CXR	Chest X-Ray
DBP	Diastolic Blood Pressure
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS

DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked Immunosorbent Assay
EPT	End Point Titers
ER	Emergency Room
FDA	Food and Drug Administration
Flu-PRO Survey Instrument	Influenza Patient Reported Outcome
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
g/dL	Grams per Deciliter
GMFR	Geometric Mean Fold Rise
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
H1N1pdm09	A/California/04/2009 (H1N1)-like Influenza A Virus
HA	Hemagglutinin
HAI	Hemagglutinin Inhibition Test
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HR	Heart Rate
iADL	Instrumental Activities of Daily Living
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor

IV	Intravenous
K	Potassium
Kg/m^2	Kilogram per Square Meter
LAR	Legally Authorized Representative
LDH	Lactate Dehydrogenase
LID	Laboratory of Infectious Diseases at NIAID
M	Molar
MAD	Mucosal Atomization Device
mg	Milligram
mg/dL	Milligrams per Deciliter
mL	Milliliter
mm	Millimeter
mm^3	Cubic Millimeter
mm Hg	Millimeter of Mercury
MMID	Mild-to-Moderate Influenza Disease
MN	Microneutralization
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
N	Number (typically refers to subjects)
Na	Sodium
NA	Neuraminidase
NAI	Neuraminidase Inhibition
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NLF	Nasal Lavage Fluid
NP	Nasopharyngeal
NSAID	Non-Steroidal Anti-inflammatory Drug
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cells
PE	Physical Exam
PI	Principal Investigator

PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
RR	Respiratory Rate
RT-PCR	Reverse Transcription – Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SBP	Systolic Blood Pressure
SE	Standard Error
sIgA	Secretory Immunoglobulin A
SMC	Safety Monitoring Committee
SpO_2	Saturation of Peripheral Oxygen
$TCID_{50}$	Median Tissue Culture Infective Dose
THC	Tetrahydrocannabinol
UA	Uric Acid
ULN	Upper Limit of Normal
US	United States
VTEU	Vaccine and Treatment Evaluation Units
WBC	White Blood Cell
wt	Wild-type

PROTOCOL SUMMARY

Title:	A Controlled Human Infection Study of Influenza A/Bethesda/MM2/H1N1 Virus (A/California/04/2009/H1N1-like) in Healthy Subjects to Assess the Effect of Pre-Existing Immunity on Symptomatic Influenza Virus Infection.
Phase:	1 (Controlled human infection study)
Population:	Up to 80 healthy adult subjects between the ages of 18 to 49 years of age inclusive, who are in good health and meet eligibility criteria.
Number of Sites:	Four domestic vaccine and treatment evaluation unit (VTEU) sites.
Study Duration:	Approximately 180 days (from time of study activation to last subject's last study day).
Subject Participation Duration:	Approximately 120 days.
Estimated Time to Complete Enrollment:	Approximately 60 days
Description of Study Product:	Influenza A/Bethesda/MM2/H1N1 virus at a total dose of 2 mL of approximately $5x10^6$/mL tissue culture infective dose ($TCID_{50}$). • Route of administration: Intranasal • Volume: 1 mL per nostril (2 mL dose total)
Design of the Study:	This is a study of a reverse-engineered, GMP grade, antiviral-sensitive, influenza A/Bethesda/MM2/H1N1 virus (A/California/04/2009/H1N1-like) infection to assess the effect of pre-existing immunity on clinical and immunological responses. Up to 80 healthy adult subjects will undergo intranasal inoculation with A/Bethesda/MM2/H1N1 virus, and their clinical manifestations, viral shedding and immunological responses will be characterized.

Table 1: Study Objectives and Outcome Measures

Primary Objective	**Primary Outcome Measure**
• To evaluate the association of symptomatic RT-PCR-positive influenza virus infection post-challenge and pre-existing HAI antibody titers	• Baseline A/Bethesda/MM2/H1N1 hemagglutination inhibition (HAI) antibody GMT association with development of MMID post-challenge, with MMID defined as presence of both of the following assessed through Day 8: 1. Viral shedding detected by any approved positive RT-PCR test from NP swab, **and** 2. Any one or more of the following symptoms or signs or laboratory findings, as related to the study agent; Arthralgia, Chest tightness, Chills, Conjunctivitis, Nasal congestion, Sinus Congestion, Coryza, Decreased appetite, Diarrhea, Dry Cough, Dyspnea/Shortness of Breath, Fatigue/Tiredness, Fever (>38.0°C), Headache, Lymphopenia (<1000 cells/mL), Myalgia, Nausea, Oxygen Saturation Decrease by ≥3% from baseline, Productive Cough, Rhinorrhea, Sore Throat, and Sweats.
Secondary Objectives	**Secondary Outcome Measures**
• To describe viral recovery by quantitative RT-PCR from study subjects at baseline and post-challenge	• Frequency, timing, magnitude, and duration of viral shedding in NLF or NP swab collected daily using quantitative RT-PCR from baseline (Day -2 or -1) and daily from Day 2 through Day 8
• To describe serum HAI and MN antibody responses post-challenge in healthy subjects by infection status	• Baseline and post-challenge HAI and MN antibody GMTs from serum measured at baseline (Day -2) and at Days, 8, 29, and 61 by infection status (RT-PCR-confirmed symptomatic, RT-PCR-confirmed asymptomatic, RT-PCR-negative symptomatic) • Percentage of healthy subjects achieving HAI and MN seroconversion (defined as either a pre-challenge titer <1:10 and a post-challenge titer ≥1:40 or a pre-challenge titer ≥1:10 and a minimum four-fold rise in post-challenge

	antibody titer) from serum measured at baseline (Day -2) and on Days 8, 29, and 61
• To evaluate the association of asymptomatic RT-PCR-positive influenza virus infection (viral shedding) post-challenge and pre-existing HAI antibody titers	• Baseline A/Bethesda/MM2/H1N1 HAI antibody GMT measured at baseline (Day -2) association with asymptomatic viral shedding by RT-PCR through Day 8
• To evaluate the association of symptomatic RT-PCR-negative status post-challenge and pre-existing HAI antibody titers	• Baseline A/Bethesda/MM2/H1N1 HAI antibody GMT measured at baseline (Day -2) association with development of any Flu-PRO symptoms post-challenge without influenza virus detection through Day 8
• To determine the frequency of serious adverse events (SAE) post-challenge	• Frequency of serious adverse events (SAE) post-challenge through the inpatient stay • Frequency of serious adverse events (SAE) post- inpatient discharge through the duration of the study (approximately three months post-challenge)
Exploratory Objectives	**Exploratory Outcome Measures**
• To describe serum NAI antibody responses post challenge in healthy subjects by infection status	• Baseline and post-challenge NAI antibody GMTs from serum measured at baseline (Day -2) and at Days, 8, 29, and 61 by infection status (RT-PCR-confirmed symptomatic, RT-PCR-confirmed asymptomatic, RT-PCR-negative symptomatic) • Percentage of healthy subjects achieving NAI seroconversion (defined as either a pre-challenge titer <1:10 and a post-challenge titer ≥1:40 or a pre-challenge titer ≥1:10 and a minimum four-fold rise in post- challenge antibody titer) from serum measured at baseline (Day -2) and on Days 8, 29, and 61
• To evaluate the association of symptomatic RT-PCR-positive influenza virus infection, asymptomatic RT-PCR-positive influenza virus infection (viral shedding only) and symptomatic RT-PCR-negative illness and pre-existing neuraminidase inhibition (NAI) antibody titers	• Baseline A/Bethesda/MM2/H1N1 neuraminidase inhibition (NAI) antibody GMT measured at baseline (Day -2) association with infection status through Day 8 by symptom/shedding status

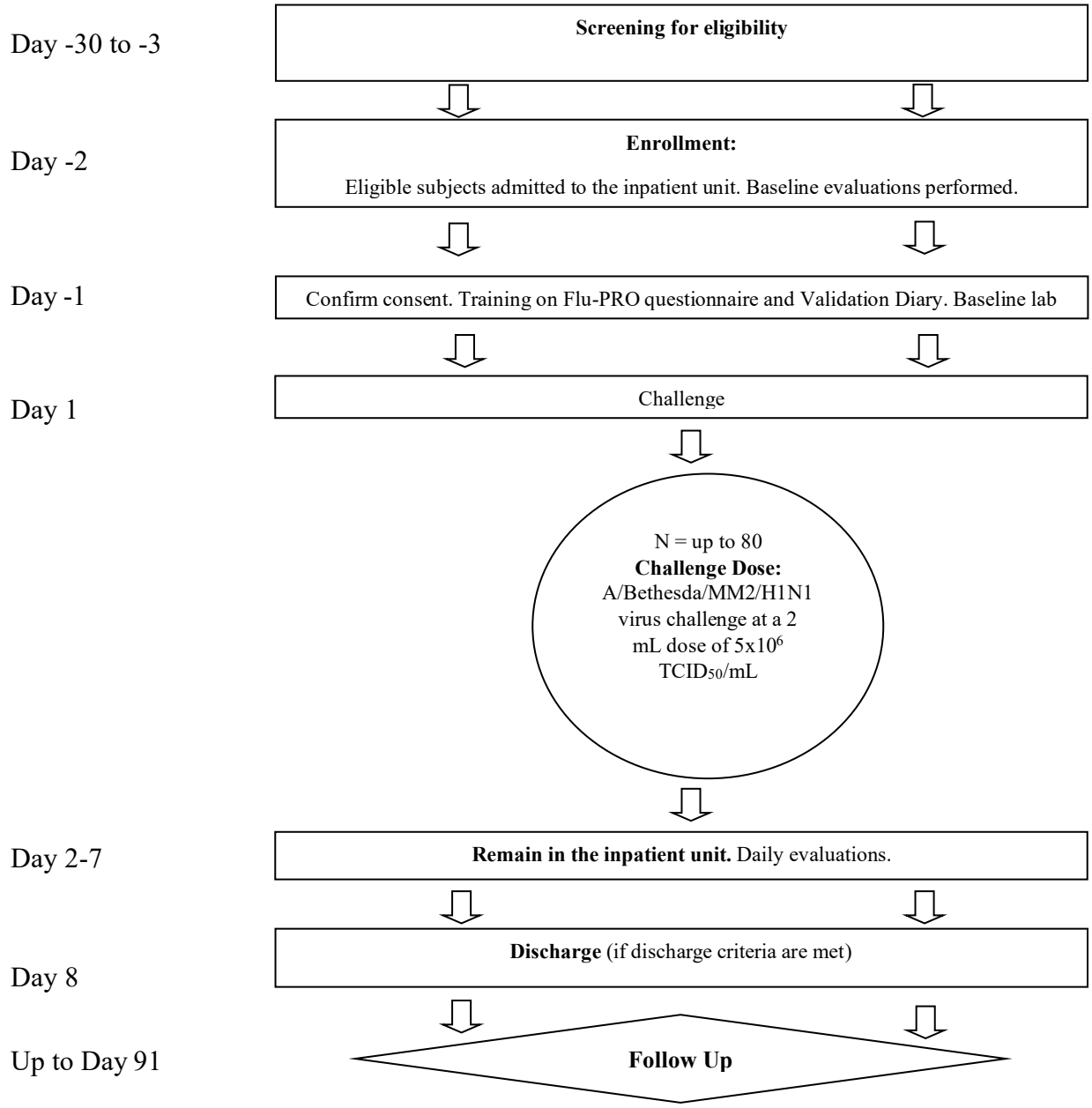
• To evaluate mucosal sIgA immune response, at baseline and post-challenge	• Positive, GMT and GMFR responses for A/Bethesda/MM2/H1N1-specific sIgA, nonnormalized and normalized for total IgA content, in NLF at baseline (Day -2), and Days 8, 29, and 61
• To evaluate serum and mucosal cytokine and chemokine responses at baseline and post-challenge	• Determine A/Bethesda/MM2/H1N1 induced cytokines and chemokines in serum at baseline (Day -2), and Days 2, 4, 6, 8, and 15 • Determine A/Bethesda/MM2/H1N1 induced cytokines and chemokines in NLF by study arm at baseline (Day -2), and approximately on Days 2, 4, 6, 8, and 15
• To assess the effects of age, sex, baseline antibody titers and prior receipt of seasonal influenza vaccine on symptomatic RT-PCR-positive influenza infection post-challenge	• Evaluate the association between baseline A/Bethesda/MM2/H1N1 hemagglutination inhibition (HAI) GMT, age, sex, and receipt of seasonal influenza vaccine in the previous year, and the development of MMID post-challenge through Day 8 by multivariate logistic regression
• To compare the clinical features of symptomatic RT-PCR-positive and RT-PCR-negative illness post-challenge	• Self-report of clinical symptoms and their severity as measured by component question in Flu-PRO Survey Instrument by post-challenge day and infection status
• To explore alternative case definitions for symptomatic RT-PCR-positive influenza virus infection	• The combination of symptoms and symptom severity as determined by self-report using the Flu- PRO instrument will be evaluated to develop clinical case definitions which optimize sensitivity, specificity, and both for influenza virus infection among subjects with evidence of RT-PCR-positive influenza virus infection post-challenge
• To evaluate the plasmablast response post-challenge in a subset of study subjects	• Determine the kinetics of A/Bethesda/MM2/H1N1-specific plasmablasts, their specific-antibody (*e.g.*, anti-H1, -N1 and -H1N1 virus) production and isotype (*e.g.*, IgG, IgA) using an enzyme-linked immunospot (ELISpot) assay in PBMCs on Days 4, 6, and 8 in a subset of subjects
• To describe systemic transcriptional responses post-challenge in a subset of study subjects	• Determine changes in peripheral blood gene expression profiling based on sequencing on

	Days 2, 4, 8, and 15 relative to baseline (Days -2 and 1 pre-challenge) in a subset of subjects
• To describe mucosal and systemic human immune cells in blood and NLF at baseline and post-challenge in a subset of study subjects	• Determine the frequency, duration, timing and phenotypic characteristics of immune cells in the blood of subjects at baseline (Day -2) and at approximately on Days 4, 6, 8, 15, 29, and 61 in a subset of subjects • Determine the frequency, duration, timing and phenotypic characteristics of immune cells in the NLF in subjects at baseline (Day -2) and Days 2, 4, 6 and 8 in a subset of subjects
• To describe anti-HA-stalk antibody titer at baseline and post-challenge	• For HA-stalk specific response, the GMTs and GMFR to group 1 stem domains will be measured at baseline (Day -2), and at approximately on Days 29 and 61
• To assess the association of human leukocyte antigen (HLA) class I and II alleles with clinical, immune and viral responses	• Determine the frequency of HLA class I and II alleles as measured by genetic testing. • To assess the association of subject HLA class I and II alleles with development of MMID post-challenge through Day 8, pre-existing immune status, and magnitude and breadth of the elicited immune responses post-challenge
• To evaluate cellular immune response at baseline and post-challenge	• Summarize the data on systemic and mucosal cytokines and chemokines induced by challenge and define their possible role in the process by which influenza exposure leads to infection, disease outcomes, and seroconversion at baseline and daily through Day 8 • Frequency of influenza-specific B cell subsets in circulation (PBMC) at baseline (Day -2), and at approximately on Days 6, 8, 15, 29 and 61 • Magnitude of influenza-specific T cell responses in circulation (PBMC) to conserved T cell epitopes and inactivated virus at baseline (Day -2), and at approximately on Days 4, 6, 8, 15, 29, and 61

Table 2: Study Intervention

Sample size	Intervention
80	Influenza A/Bethesda/MM2/H1N1 Challenge Virus 2 mL of approximately $5x10^6$ $TCID_{50}$/mL

Figure 1: Schematic of Study Design



1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

The high morbidity and mortality associated with both pandemic and seasonal influenza, the continued threat of highly pathogenic avian influenza infections, the development and global spread of antiviral resistance, and the inadequacy of current influenza vaccines makes influenza a high research priority. Although prior influenza research has addressed numerous aspects of viral biology and pathogenesis, many questions remain unanswered. Advancing basic, translational and clinical research on influenza will inform the development of new and improved diagnostics, therapeutics, and vaccines [1].

The natural history, pathogenesis, and immunology of human influenza have not been well characterized and cannot be adequately studied in animal models. Even the best models such as those in pigs and ferrets, although useful, have demonstrated limitations in their relevance to human disease. Influenza human challenge models offer a unique opportunity to further study influenza pathogenesis and to overcome some of the limitations associated with animal models and/or natural history studies in humans. These models are conducted in a well-controlled challenge setting in which virus exposure, infection rate, virus shedding, and sampling are being monitored in real-time. Such parameters are challenging to control in a "real world" setting.

Previous human challenge studies conducted in the US during the 1970s, 1980s, and 1990s advanced our understanding of the natural history, clinical characteristics, and immune responses following infection [2-14]. Such studies were also used to assess the efficacy of vaccines and were critical for advancing the clinical development of influenza antivirals. Although these studies have provided important information, their generalizability to current questions is limited due to the scope of the studies, the scientific techniques that were available at the time, the evolution of circulating virus strains and population immunity, and advances in antiviral and vaccine development [2-14].

Ensuring subject safety is a paramount concern for any clinical study, particularly human challenge studies. In the early 2000s, influenza human challenge studies in the US were stopped due to a clinical event occurring after a human challenge study that was investigating the use of the antiviral peramivir as a prophylactic agent [15]. The subject was a 21-year-old, previously healthy man with no prior cardiac history. Following receipt of peramivir and infection with an influenza B challenge strain, he had asymptomatic ECG changes (described as new T wave inversions of leads II, aVF, and v4-6) on day 4 of the study. Repeat ECG at day 15 had returned to baseline, and he had no cardiac symptoms or cardiac enzyme (CPK) elevation. He then traveled to Indonesia for 2 weeks and became ill with an upper respiratory infection. When he returned to the US, he had an echocardiography performed at 51 days post influenza challenge, since he had had initial ECG changes post-infection. The echocardiogram showed reduced ejection fraction, with left ventricular enlargement. The man remained asymptomatic. Over the next 5 months, repeat echocardiograms showed gradual improvement and return to normal ejection fraction. The cause of the dilated cardiomyopathy was not ascertained but deemed

unlikely to be due to the study drug and also deemed unlikely to be due to the challenge virus [15, 16].

As a result of the clinical event described above, influenza challenge studies were not performed in the US for many years. A large and thorough review of 56 different influenza challenge studies, conducted in 2008 [4], confirmed that infection from an influenza challenge stock induced only mild disease, with one third of subjects having a fever and one fifth of subjects developing lower respiratory symptoms. Previous influenza challenge studies have demonstrated that the required dosing differs depending on the viral strain, subtype, and administration method (Table 3) [17]. Influenza challenge studies in Europe continued to be performed [18].

Since 2012, the Laboratory of Infectious Diseases (LID) at NIAID has been working to re-establish a controlled influenza A/H1N1 challenge model in healthy subjects in the US. The first dose-ranging study of 49 healthy subjects using a Good Manufacturing Practice (GMP) influenza A (H1N1pdm09) challenge strain demonstrated that mild to moderate influenza illness could be induced in 70% to 80% of healthy subjects and mimicked natural infection both clinically and immunologically [19]. Clinical symptoms of influenza occurred post-challenge with doses ranging from 10^3 to 10^7 $TCID_{50}$ but were most prevalent at the 10^6 and 10^7 $TCID_{50}$ doses (Table 4).

NIAID's second challenge study at LID using the H1N1 model evaluated anti-hemagglutinin (HA), anti-neuraminidase (NA) and anti-HA stalk antibodies as correlates of protection in H1N1 influenza illness in 74 healthy subjects, finding that while higher HAI titers are indicative of some protection, NAI titers may be more predictive of protection from influenza infection and reduced disease [20, 21]. These studies have led to a number of ongoing laboratory studies using samples collected to address questions of intra-host evolution, gene expression, and immune response, many of which are expected to be published in the coming months. In clinical studies the model is now being implemented to test new therapeutics and vaccines (NCT02371668 and NCT03845231). This virus has been used in 3 published trials and 1 unpublished trial to date in approximately 400 participants. No significant safety issues have been identified, no severe or complicated cases of influenza have occurred, and no transmission events outside of the study have been observed [19, 20, 22].

These studies helped to define a clinical endpoint termed "mild-to-moderate influenza disease" (MMID) which included evidence of influenza A virus shedding by RT-PCR and any of a number of standardized signs, symptoms, and laboratory findings consistent with clinical influenza illness. MMID is assessed using a validated, patient-reported outcome (PRO) measure to standardize assessment of influenza (flu) symptoms in clinical research, i.e. Flu-PRO Survey Instrument [23]. MMID is defined as influenza A virus shedding plus any one or more of the following symptoms or signs: arthralgia, chest tightness, chills, conjunctivitis, nasal congestion, sinus congestion, coryza, decreased appetite, diarrhea, cough, dyspnea/shortness of breath, fatigue/tiredness, fever (>38.0°C), headache, lymphopenia (<1000 cells/mL), myalgia, nausea, oxygen saturation decrease by ≥3% from baseline, rhinorrhea, sore throat, and sweats. The license agreement with Leidos Biomedical Research to use the Flu-PRO Survey Instrument requires that an additional Validation Diary comprised of nine questions be completed by subjects and that data from both instruments be provided to Leidos for further Flu-PRO

quantitative validation exercises. Both Flu-PRO Survey Instrument and the Validation Diary are in Appendix D.

One of the key components and gap-filling measures in NIAID's strategic plan for universal influenza vaccine development is to expand the capacity to conduct human challenge studies with relevant influenza challenge viruses [1]. Human challenge models provide a powerful tool to assess prevention and treatment options for influenza in a shorter time frame, with a smaller number of subjects and against a known virus strain. Due to the known time of infection, they allow for targeted collection of clinical samples, including prior to infection and in short time frames following inoculation. Human challenge models may be particularly useful to evaluate novel influenza vaccines that do not target the HA head region, and therefore cannot be judged based on current regulatory criteria for current inactivated influenza vaccines. Through collaboration with both other academic and industry partners, these models have and will continue to serve as an important resource in the influenza research community.

2.2 Scientific Rationale

2.2.1 Purpose of Study

This proposed study will establish a H1N1 influenza virus controlled human infection (CHI) model at the NIAID Division of Microbiology and Infectious Diseases (DMID)-sponsored VTEU clinical study sites. The model has been developed and used by NIAID Division of Intramural Research. The proposed study will expand US research capacity to perform influenza CHI studies to advance the understanding of influenza pathogenesis and novel vaccine research and development.

The scientific goal of this study is to conduct an influenza CHI study in healthy adults to evaluate the association of pre-existing HAI titers and the development of symptomatic RT-PCR-positive influenza virus infection. The study will build upon the NIH Clinical Center's influenza CHI model with influenza A/California/04/2009 (H1N1)-like virus [19]. A CHI of up to 80 healthy subjects will be performed using a total dose of approximately 1 x 10^7 $TCID_{50}$, which has been shown to induce 60% or higher rates of MMID in individuals who had pre-challenge HAI titers of ≤1:40 [19]. Additional study objectives will assess clinical manifestations, viral shedding and immunological responses to the challenge virus.

Table 3: Doses Used in Previous Challenge Studies in Humans Since 1985

	Reference	Dose (log_{10} TCID $_{50}$)	Proportion of Subjects*		Mean Peak Titer Virus Shed (log_{10} $TCID_{50}$ ± SE)	Mean Duration of Shedding (Days ± SE)	Proportion of Subjects with		
			Infected†	Shedding Virus			Systemic Febrile Illness	Respiratory Illness	Lower Respiratory Illness‡
H1N1	**A/California/10/78**								
	Treanor *et al*. (1987)	4.5	8/9	8/9	1.98	NR	0/9	5/9	NR
	[24]	4.5	8/8	8/8	2.4±0.4	3.6±0.6	2/8	3/8	NR
	Treanor *et al*. (1990)	4.0	11/15	11/15	3.8±1.6	6.8±1.5	5/15	3/15	1/15
	[25]								
	Clements *et al.* (1986)								
	[26]								
	A/Hong Kong/123/77								
	Betts *et al*. (1985) [27]	4.2	15/16	15/16	4.2±2.1	4.7±2.1	8/16	5/16	NR
	A/Kawasaki/9/86								
	Youngner *et al.* (1994)	7.0	NR	10/14	2.2±0.4	NR	3/14	6/14	NR
	[28]	7.0	16/16	16/16	2.8±0.5	NR	1/16	NR	2/16
	Hayden *et al.* (1994)								
	[29]								
	A/Texas/1/85								
	Sears *et al.* (1988) [30]	6.4	26/28	26/28	3.1±1.9	4.5±2.4	8/28	11/28	NR
	Sears *et al.* (1987) [31]	6.7	20/22	18/22	3.0±1.9	3.5±1.7	5/22	9/22	1/22
	A/Texas/91								
	Hayden *et al*. (1996)	5.0	26/26	24/26	3.2±2.1	2 (0-6)§	10/26	21/26	7/26
	[32]								
H3N2	**A/Bethesda/1/85**								
	Treanor *et al.* (1990)	4.5	5/5	4/5	2.4±0.6	3.0±1.0	2/5	2/5	NR
	[25]	7.0	10/10	10/10	4.2±1.4	5.9±1.3	3/10	1/10	NR
	Sears *et al.* (1988) [30]	7.1	18/19	18/19	3.5±0.5	6.5	NR	11/19	NR
	Reuman *et al.* (1989)								
	[33]								
	A/England/40/83								
	Al-Nakib *et al.* (1986)	4.1	31/31	30/31	4.0	NR	NR	17/31	NR
	[34]								
	A/Korea/1/82								
	Snyder *et al.* (1988) [35]	6.0	12/14	12/14	3.4±0.5	5.1±0.3	1/14	5/14	NR
	A/Los Angeles/2/87								
	Clements *et al.* (1992)	7.0	22/24	16/24	1.5±0.3	2.6±0.4	4/24	11/24	NR
	[36]								
	A/Washington/897/80								

	Reference	Dose (log_{10} $TCID_{50}$)	Proportion of Subjects*		Mean Peak Titer Virus Shed (log_{10} $TCID_{50}$ ± SE)	Mean Duration of Shedding (Days ± SE)	Proportion of Subjects with		
			Infected†	Shedding Virus			Systemic Febrile Illness	Respiratory Illness	Lower Respiratory Illness‡
	Clements *et al.* (1986) [26]	6.0	25/27	22/27	3.8±1.6	4.3±2.0	10/27	7/27	3/27
B	**B/Ann Arbor/1/86** Clements *et al.* (1990) [37]	7.0	10/12	8/12	4±0.7	5.4±1	2/12	5/12	NR
	B/Texas/1/84 Keitel *et al.* (1990) [38]	7.1	6/6	6/6	2.2±0.7	5.0±0.6	NR	3/6	NR
	B/Yamagata/16/86 Treanor *et al.* (1993) [39]	7.0	10/10	7/10	2.3	NR	3/10	7/10	2/10

Adapted from a previous publication [17]. NR = Not Reported; * Number with finding / number evaluated; † Virus shedding, serum antibody response, or both; ‡ Reported as "lower respiratory illness" or "cough"; § Expressed as median (range).

Table 4: Dose Escalation Results with A/Bethesda/MM2/H1N1 challenge strain

Dose	Male	Female	Total	Symptoms	Viral Shedding	Both (MMID)	Four-fold Rise in HAI Titer
10^3 $TCID_{50}$	3	2	5	2 (40%)	0	0	1 (20%)
10^4 $TCID_{50}$	4	0	4	2 (50%)	0	0	0
10^5 $TCID_{50}$	2	3	5	4 (80%)	1 (20%)	1 (20%)	1 (20%)
10^6 $TCID_{50}$	10	9	19	17 (89%)	9 (47%)	9 (47%)	16 (84%)
10^7 $TCID_{50}$	9	4	13	11 (85%)	10 (77%)	9 (69%)	11 (85%)
Total	28	18	46	36 (78%)	20 (43%)	19 (41%)	29 (63%)

Data are presented as N (%).

Abbreviations: HAI, hemagglutinin inhibition; MMID, mild-to-moderate influenza disease; $TCID_{50}$, median tissue culture infective dose.

2.2.2 Study Population

The study population will be healthy subjects (male and non-pregnant, non-breastfeeding females) between the ages of 18 to 49 inclusive drawn from the general communities of each study site. They must be non-habitual smokers, including non-habitual smokers of marijuana or e-cigarettes. Subjects must not have medical conditions at high risk for severe complications of influenza virus infection, as defined by the US Centers for Disease Control and Prevention (CDC) (Appendix C). Up to 80 healthy subjects will undergo A/Bethesda/MM2/H1N1 virus CHI and their clinical manifestations, viral shedding, and immunological responses will be characterized.

2.3 Potential Risks and Benefits

2.3.1 Common Symptoms of Influenza Illness

- Common signs or symptoms of influenza illness include:
- Body aches or pains
- Chest congestion
- Chest tightness
- Chills
- Congested or stuffy nose
- Coughing
- Diarrhea
- Difficulty swallowing
- Eyes sensitive to light
- Fatigue
- Felt dizzy
- Fever
- Head congestion
- Headache
- Lack of appetite

- Nausea
- Runny or dripping nose
- Scratchy or itchy throat
- Shivering
- Sinus pressure
- Sleeping more than usual
- Sneezing
- Sore or painful eyes
- Sore or painful throat
- Stomach ache
- Sweating
- Teary or watery eyes

2.3.2 Potential Risks

Complications or severe signs or symptoms of influenza illness, which are unlikely to occur in healthy subjects who participate in this study, include:

- Pneumonia
- Dehydration
- Difficult breathing
- Severe bronchitis
- Myocarditis
- Myocardial infarction
- Pericarditis
- Guillain Barré Syndrome/ Bell's Palsy
- Transverse myelitis
- Encephalitis
- Sinus infections
- Ear infections
- Worsening of chronic health conditions
- Admission to an intensive care unit (ICU)
- Hypoxemia/respiratory failure
- Arrhythmia/cardiac arrest
- Death

There may be other risks, discomforts, or side effects that are unknown at this time.

Risks of Nasal Lavage or Nasopharyngeal Swabs

Obtaining a nasal lavage fluid or a nasopharyngeal swab can cause discomfort in the nares, a gag reflex, epistaxis, watery eyes, or coughing at the time of collection.

Risks of Blood Draw

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the draw site for several minutes. Drawing blood may also cause infection. The use of sterile technique will make infection at the site where blood will be drawn extremely unlikely. Risks for blood drawn may include anemia, however the risk is low since the total amount drawn over the entire study period is about the same as a liter of blood (i.e. 525 mL). The blood volumes anticipated for specimen collection are listed in Appendix B.

Risks of Chest Radiograph (CXR)

According to the United Nations Scientific Committee on the Effects of Atomic Radiation, a single posterior-anterior chest radiograph (CXR) exposes the subjects to about 0.05 mSv, which is about the radiation dose people are exposed to naturally over the course of 10 days. Subjects in our study will have one CXR at screening (Days -30 to -3), and if deemed medically necessary as follow-up of an Adverse Event. Female subjects will a serum pregnancy test, and an additional urine pregnancy test if more than 7 days have passed since the serum test, before CXR.

Risks of Electrocardiogram (ECG)

The electrodes of an electrocardiogram (ECG) may feel cold when applied; in rare cases, a rash or skin irritation develops where the patches are placed. This type of irritation usually resolves by itself, but topical medication is occasionally required. Subjects in our study will have one ECG at screening (Days -30 to -3), a repeat ECG at study Day 6, and additional ECGs if deemed medically necessary.

Risks of Xofluza (Baloxavir Marboxil) Antiviral

Adverse events reported in at least 1% of adult and adolescent subjects treated with baloxavir marboxil included diarrhea (3%), bronchitis (2%), nasopharyngitis (1%), headache (1%) and nausea (1%) [40]. Influenza A viruses with treatment-emergent amino acid substitutions at positions associated with reduced susceptibility to baloxavir marboxil in cell culture have been observed in clinical studies. It is theoretically possible that baloxavir marboxil treatment can cause treatment-emergent baloxavir marboxil resistant viruses. The overall incidence of treatment-emergent amino acid substitutions associated with reduced susceptibility to baloxavir marboxil in two prior clinical trials was 2.7% (5/182) and 11% (39/370) [40].

Risks of Delaying Influenza Vaccination Until 60 Days Post-challenge

In the US, routine annual influenza vaccination is recommended for all persons, with an emphasis placed on vaccination of high-risk groups and their caregivers. In this study, subjects are asked to delay 2019-2020 influenza season vaccination through 60 days post-challenge. If the influenza season begins before this time, the subject is at risk of developing influenza illness.

Symptoms of influenza are described in 2.3.1 and the potential risks of influenza are described above in this Section.

Risks of Genetic Testing

Genetic data and health information may be stored and shared with other researchers through a controlled-access repository, such as dbGaP. There may be a risk that information resulting from research genetic testing could be misused for discriminatory purposes. However, state and federal laws provide some protections against genetic discrimination. Researchers who will have access to genetic information but will take measures to maintain the confidentiality of the information. HLA genetic testing will be performed for research purposes only and will not be performed by a CLIA certified laboratory. Therefore, the results of HLA testing will not be shared with subjects.

2.3.3 Potential Benefits

There is no direct benefit to the subjects. There is potential benefit to society and future patients resulting from insights gained from participation in this study.

3 STUDY OBJECTIVES AND OUTCOME MEASURES

Refer to Table 1 Study Objectives and Outcome Measures

4 STUDY DESIGN

This is a CHI study of Influenza A/Bethesda/MM2/H1N1 virus to assess clinical response, immunological response, and safety. The study population will be healthy subjects (male and non-pregnant, non-breastfeeding females) between the ages of 18 to 49, inclusive. Up to 80 subjects will receive A/Bethesda/MM2/H1N1 virus CHI. Their clinical manifestations, viral shedding, and immunological responses will be characterized.

Influenza A/Bethesda/MM2/H1N1 virus CHI will be performed using a 2 mL dose of approximately $5x10^6$ $TCID_{50}$/mL, which has been shown to induce 60% or higher rates of MMID [19]. The primary objective of the study will be to evaluate the association of MMID post-challenge and pre-existing virus-specific HAI titers in healthy subjects. MMID is defined as the presence of both of the following, assessed through day 8:

- Viral shedding detected by any approved positive RT-PCR assay from a NP swab, **and**
- Any one or more of the following symptoms or signs or laboratory findings, as related to the study agent; Arthralgia, Chest tightness, Chills, Conjunctivitis, Nasal congestion, Sinus Congestion, Coryza, Decreased appetite, Diarrhea, Dry Cough, Dyspnea/Shortness of Breath, Fatigue/Tiredness, Fever (>38.0°C), Headache, Lymphopenia (<1000 cells/mL), Myalgia, Nausea, Oxygen Saturation Decrease by ≥3% from baseline, Productive Cough, Rhinorrhea, Sore Throat, and Sweats.

During Study Days -30 to -3 (Screening Period), subjects will provide informed consent to participate in this study. They will undergo a review of medical history, physical examination by a study physician, and screening laboratory tests. During this period, subjects will also have a Posterioranterior (PA) and Lateral chest X-ray (CXR), and a baseline 12-lead ECG.

After enrollment, subjects will be admitted to an inpatient challenge unit (Day -2). Each study site will perform CHI on up to 20 subjects unless otherwise directed by DMID to meet the total number to be enrolled. This may be done in one or two cohorts. Backup subjects will be admitted into the inpatient unit to ensure sufficient number of volunteers undergo CHI, given the potential for drop-outs, presence of new exclusionary criteria, and/or pre-challenge evidence of viral respiratory infection among subjects. The number of backups will depend on site-specific considerations, such as anticipated drop-out rates, anticipated development of new exclusionary criteria, and respiratory virus circulation in the community. A review of eligibility criteria, and baseline clinical assessments, safety blood tests, influenza virology, and immunology tests will be performed and the subjects will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the inpatient unit before CHI. Subjects must have no evidence of a respiratory viral infection as determined by multiplex respiratory virus RT-PCR assay testing prior to CHI (positive results prior to CHI will exclude the subject from the challenge, and additional subjects may serve as replacements). On Day -1, subjects will be

trained on the influenza patient related outcomes Flu-PRO Survey Instrument and Validation Diary[41].

On Day 1, eligibility and clinical status will be reviewed. If criteria for proceeding with challenge are met, subjects will receive A/Bethesda/MM2/H1N1 CHI. One mL of A/Bethesda/MM2/H1N1 will be delivered in each nostril of a recumbent subjects per a study SOP. Backups will be discharged from the inpatient unit prior to challenging other subjects if they are not needed to replace ineligible subjects or subjects declining to proceed with CHI.

After CHI, subjects will complete daily self-assessments using the Flu-PRO Survey Instrument [41] and Validation Diary through 14 days post-challenge to generate a symptom severity score. Subject clinical status will be monitored closely. Safety labs will be performed at scheduled times. Subjects will undergo scheduled blood draws, nasal lavages, and nasal swab collections for immunology and virologic laboratory testing. Immunologic evaluations will include assessment of antibody and cytokine response, T and B cell responses, and systemic transcriptional responses. Quantitative and qualitative evaluation of influenza will be done after CHI on Days 2 through 8. Subjects will also undergo daily qualitative evaluations for evidence of influenza by multiplex respiratory virus RT-PCR assay. Subjects will remain blinded to the results of their respiratory virus testing until they meet discharge criteria. An ECG will be performed at Day 6 on all subjects, and as clinically indicated at any other time.

Following CHI, subjects will remain in the inpatient unit for a minimum of seven additional days and will be discharged only if 1) they have two consecutive negative influenza tests (that are performed on days 7 and 8) for influenza A using a multiplex respiratory virus assay (BIOFIRE® FILMARRAY® or Luminex xTAG®), AND 2) have been afebrile (<38.0°C), have a room air $SpO_2 \geq 95$ and have been clinically and hemodynamically stable for at least 48 hours. Discharge evaluation and criteria will be further described in a study SOP. In addition, subjects may have residual and/or resolving symptoms of influenza illness. It is expected that most subjects will shed the challenge virus for three to five days post exposure.

If subjects are still shedding virus on Day 8, they will remain in the challenge unit, be offered one dose of baloxavir marboxil (a full treatment course), and they will continue to have nasopharyngeal (NP) swabs tested daily until they have two negative influenza tests performed on consecutive days and meet clinical discharge criteria described above.

Any subject who develops serious related or unrelated signs or symptoms during inpatient stay will receive appropriate and as clinically necessary escalation of care (*e.g.*, cardiologist consultation for cardiac symptoms or transfer for evaluation at the Emergency Department). Any subjects who experience complications or sequelae due to CHI will be followed until resolution and/or stable, and they will be referred for appropriate specialized care as clinically necessary. SOPs will be utilized to standardize evaluation and care for select known complications of influenza virus infection. All subjects will be followed for approximately 90 days post-challenge. Additional follow-up will be performed at in-person Visits 9 (Day 15), 10 (Day 29) and 11 (Day

61). Subjects will also undergo scheduled blood draws and, nasal lavages for immunology and virologic laboratory testing during this follow up period. A final follow-up by telephone will occur on Visit 12 (Day 91).

5 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

5.1 Study Product Description

5.1.1 Formulation, Packaging, and Labeling

The study agent in this protocol is a biologic (live virus) and requires special storage and handling. The A/Bethesda/MM2/H1N1 human challenge virus was manufactured in certified Vero cells under Good Manufacturing Practice (GMP) conditions by Charles River Laboratories in Malvern, PA from a reverse-genetics (RG) derived virus seed stock produced by Dr. Matthew Memoli and his laboratory team in the Viral Pathogenesis and Evolution Section of the Laboratory of Infectious Diseases (NIAID). The rescued RG derived virus was passaged six times in certified Vero cells to produce the seed virus.

The final product was vialed at approximately 5 X 10^6 $TCID_{50}$/mL of Influenza A Human Challenge Virus (H1N1) in 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; L-glutamic acid, 0.0054 M). Each vial has a fill volume of 1.1 mL. The filled vials were snap frozen in a dry ice-ethanol bath and stored at -80°C ± 15°C.

Each vial will be individually labeled with the product name, description, and caution statement. The label was affixed to 2 mL cryogenic vials prior to final fill with A/Bethesda/MM2/H1N1 Virus [Charles River Laboratories] (Lot #MM001).

The Challenge virus is on an on-going stability and potency program and the most recent potency assessment performed in June 2019 is 5x10^6 $TCID_{50}$/mL. CHI will be performed using a 2 mL dose of approximately 5x10^6 $TCID_{50}$/mL virus and will be administered intranasally. Intranasal CHI will be carried out using the MAD Nasal sprayer device (Wolfe-Tory Medical, Salt Lake City, Utah) attached to a 1 mL Luer lock syringe. One mL of virus will be delivered in each nostril of a recumbent subject.

5.1.2 Product Storage and Stability

Influenza A/Bethesda/MM2/H1N1)

The human challenge virus will be stored at -80°C ± 15°C in single-use vials. Additional details of product storage and stability can be found in the MOP. Preparation and dosing of the agent must take place within three hours of removing the virus from the freezer. The MAD Nasal sprayer device (Wolfe-Tory Medical, Salt Lake City, Utah) will be stored at room temperature (15 -30°C).

5.2 Acquisition/Distribution

Influenza A/Bethesda/MM2/H1N1

The human challenge virus will be stored and distributed by the DMID Clinical Material Services (CMS) to designated VTEU sites. The study agent will be handled by study personnel and administered only in the inpatient unit. The MAD Nasal sprayer device (Wolfe-Tory Medical, Salt Lake City, Utah) will be obtained and distributed by the DMID-Clinical Material Services (CMS) to designated VTEU sites.

5.3 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Investigational Product

Influenza A/Bethesda/MM2/H1N1

All steps in preparing the syringes and attaching MAD nasal atomizer to each syringe should be performed with careful sterile technique in a biosafety cabinet BSL Level 2 laboratory.

CHI will be performed using a 2 mL dose of approximately $5x10^6$ $TCID_{50}$/mL virus and will be administered intranasally. Intranasal CHI will be carried out using the MAD Nasal sprayer device (Wolfe-Tory Medical, Salt Lake City, Utah) attached to a 1 mL syringe. Two vials will be needed per subject. The time that the 2 vials are withdrawn from the freezer will be documented in the Accountability Log. Once thawed 1.0 mL of virus will be drawn from each of vial and kept on wet ice until administered. Prior to administration the syringe will be attached to the MAD nasal atomizer. The total time from freezer to administration will not exceed 3 hours. One mL of virus will be delivered in each nostril of a recumbent participant. CHI will be performed according to the study MOP.

The challenge virus is nearly identical to circulating influenza A H1N1pdm09 viruses. This virus is susceptible to FDA-approved neuraminidase inhibitors oseltamivir and zanamivir and sequencing does not indicate any mutations that confer baloxavir marboxil resistance [42, 43]. The 2009 H1N1pdm viruses are resistant to the adamantane class of influenza antivirals [43]. Full genomic sequencing was performed on the 2009 H1N1 Challenge Virus (Lot MM#001). A total of 4 nucleic acid changes post manufacturing were observed, 1 each in HA, PA, and NA, as well as one synonymous change. The three nonsynonymous changes were found as the dominant base in a double peak during sequencing along with the original wild-type (wt) nucleotide. There is no indication that the 2009 H1N1 Challenge Virus would behave any differently in humans than the wt influenza A/California/04/2009 (H1N1) virus, based on the in vitro and in vivo data.

Table 5: Study Intervention Regimen

Product Name	Dose	Route	Frequency of Administration
A/Bethesda/MM2/H1N1	2 mL of approximately $5x10^6$ $TCID_{50}$/mL	Intranasal	Challenge virus will be administered once on Day 1
$TCID_{50}$, median tissue culture infective dose			

5.4 Pre-determined Modification of Study Intervention/Investigational Product for an Individual Subject

Not applicable. DMID will not permit modification of study product dose or regimen.

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

Influenza A/Bethesda/MM2/H1N1 will be stored and shipped from the DMID contractor CMS to the Clinical Sites. Once received, Influenza A/Bethesda/MM2/H1N1 will be stored in and dispensed by the Investigational Pharmacy.

After receipt of the study products, the site principal investigator is responsible for study product distribution and disposition and has ultimate responsibility for study product accountability. The site principal investigator may delegate to the participating site's research pharmacist responsibility for study product accountability. The participating site's research pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study product(s). All study product(s), whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the participating site's study product accountability records and dispensing logs per the site monitoring plan.

Used and unused of study products will be retained until monitored and released for disposition, as applicable. This can occur on an ongoing basis for used study products.

Used study products may be destroyed in accordance with site-specific SOPs following each monitoring visit where study product accountability is monitored, and resolution of any discrepancies.

Final disposition of the unused study products will be determined by DMID and communicated to the participating sites by the DMID Clinical Project Manager.

The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained, and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the DMID monitoring contractors and is subject to inspection by a regulatory agency (*e.g.*, FDA) or the Sponsor at any time. An assigned Study Monitor will review the pharmacy records.

6 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses. No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

6.1 Eligibility Criteria

6.1.1 Subject Inclusion Criteria

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

1. Provide written informed consent prior to initiation of any study procedure.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Agree to remain an inpatient for at least seven days after challenge, AND until they have no virus shedding,[1] determined by qualitative RT-PCR for a minimum of two consecutive days post-challenge

   [1]For a minimum of seven days post-challenge (Study Day 8).
4. Healthy[2] males and non-pregnant, non-breastfeeding females[3] aged ≥18 and ≤49 years of age, inclusive, at enrollment.

   [2]Good health is defined in inclusion criteria #10.

   [3]Females subjects of childbearing potential must agree to have a serum pregnancy test at screening, a urine pregnancy test before CXR performed (if more than 7 days have passed between CXR and serum pregnancy test), and a urine pregnancy test upon admission to the inpatient unit prior to CHI, and results must be negative.
5. Women of childbearing potential[4] must agree to use or have practiced true abstinence[5] or use at least 1 acceptable primary form of contraception[6,7]

   These criteria are applicable to females in a heterosexual relationship and child-bearing potential (i.e., the criteria do not apply to subjects in a same sex relationship).

   [4]Not of child bearing potential - post-menopausal females (defined as having a history of amenorrhea for at least one year) or a documented status as being surgically sterile (hysterectomy, bilateral oophorectomy, tubal ligation/salpingectomy, or Essure® placement with history of documented radiological confirmation test at least 90 days after the procedure).

[5]*True abstinence is 100% of time no sexual intercourse (male's penis enters the female's vagina). (Periodic abstinence [e.g. calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception).*

[6]*Acceptable forms of primary contraception include monogamous relationship with a vasectomized partner who has been vasectomized for180 days or more prior to the subject receiving the influenza challenge virus, intrauterine devices, birth control pills, and injectable/implantable/insertable hormonal birth control products.*

[7]*Must use at least one acceptable primary form of contraception for at least 30 days prior to admission and at least one acceptable primary form of contraception during the remainder of the study.*

6. Non-habitual smoker[8] of tobacco, e-cigarettes or marijuana.

   [8]*Non-habitual smokers are those who smoke no more than four cigarettes, other tobacco products, e-cigarettes or marijuana in a week for more than three months and agree not to smoke cigarettes, other tobacco products, e-cigarettes and/or marijuana products during participation in the study.*

7. No self-reported or known history of alcoholism or drug use within the last 30 days and agrees to abstain from alcohol and drugs[9] for at least one week before admission and throughout the inpatient period.

   [9] *including forms of marijuana not included in criterion 6*

8. Negative drug urine toxicology result on screening (i.e., amphetamines, cocaine, and opiates) and on admission to the challenge unit (i.e., amphetamines, cocaine, opiates, **and cannabinoids**).

9. Agree not to use the listed[10] prescription or over-the-counter medications within 7 days prior to inpatient stay and through inpatient stay, unless approved by the investigator.

   [10]*Oseltamivir, zanamivir, peramivir, baloxavir marboxil, amantadine (generic) and rimantadine (Flumadine and generic), aspirin, intranasal steroids, decongestants, antihistamines, and other non-steroidal anti-inflammatory drugs (NSAIDs).*

10. In good health[11] and not have clinically significant medical, psychiatric, chronic or intermittent health conditions including those listed in Exclusion Criteria (Section 6.1.2).

    [11]*Good health, as determined by medical history, medication use and physical examination to evaluate ongoing chronic medical or psychiatric diagnoses or conditions, defined as those that have been present for at least 90 days, which would not affect the assessment of the safety of subjects or the immunogenicity of challenge. These medical diagnoses or conditions should be stable for the last 90 days (no hospitalizations, emergency room (ER) or urgent care for condition (excluding musculoskeletal conditions) and not listed in Exclusion Criteria*

(Section 6.1.2). Subjects may be on medications only if the condition or disease is stable and not deteriorating, if the medical intervention (such as device or medication) was not available during the maximal inpatient period of time, medications are not listed in the Exclusion Criteria (Section 6.1.2.) and pose no additional risk to subject safety or assessment of adverse events. This also includes no change in prescription medication, dose or frequency as a result of new symptoms or deterioration of the medical diagnosis or condition in the 90 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome (e.g., lowering of the dosage or frequency), as determined by the site principal investigator (PI) or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572, will not be considered a deviation of this inclusion criterion.

11. Does not have an ongoing symptomatic condition[12] for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan.

    [12]*e.g., ongoing fatigue without a diagnosis for symptom.*

12. Vital signs as follows: pulse is 47 to 99 beats per minute, inclusive; systolic blood pressure is 85 to 139 mmHg, inclusive; diastolic blood pressure is 55 to 89 mmHg, inclusive; SpO_2 $\geq$95%; RR$\leq$18; oral temperature is less than 100.0°F.

13. Eligibility laboratory values (WBC, Absolute Lymphocyte Count, Hgb, PLTs, ALT and Cr) are within acceptable parameters. (See Table 6)

14. Body mass index (BMI) >18.5 and <35 kg/m^2 at screening.

15. Other screening tests (ECG and CXR) are within normal reference range or not deemed clinically significant by the PI or appropriate sub-investigator[13]

    [13]*Designated clinician licensed to make medical diagnoses and listed on the Form FDA 1572*

16. Negative test for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) at screening blood draw.

17. Negative respiratory virus panel by BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or by Luminex xTAG® on Day -2, and Day -1.

6.1.2 Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Female subject who has a positive pregnancy test on screening or admission, is breastfeeding or planning to become pregnant from 30 days prior to challenge through the end of the study.

2. Presence of self-reported or medically documented significant medical or psychiatric condition(s)[14]

   [14]*Significant medical or psychiatric conditions include but are not limited to:*

   a. *Respiratory disease (e.g., chronic obstructive pulmonary disease [COPD], asthma) requiring daily medications[15] currently or any treatment of respiratory disease exacerbations (e.g., asthma exacerbation) in the last 5 years*

      [15]*Asthma medications: inhaled, oral, or intravenous (IV) corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics.*

   b. *Presence of any febrile illness or symptoms suggestive of a respiratory infection within two weeks prior to CHI.*

   c. *Significant cardiovascular disease (e.g., congestive heart failure, cardiomyopathy, ischemic heart disease) or history of myocarditis or pericarditis as an adult.*

   d. *Neurological or neurodevelopmental conditions (e.g., epilepsy, stroke, seizures, encephalopathy, focal neurologic deficits, Guillain-Barre syndrome, encephalomyelitis or transverse myelitis).*

   e. *Ongoing malignancy or recent diagnosis of malignancy in the last five years excluding basal cell carcinoma of the skin, which is allowed.*

   f. *An autoimmune disease.*

   g. *An immunodeficiency of any cause.*

   h. *A history of diabetes.*

3. Presence of immunosuppression or any medications that may be associated with impaired immune responsiveness[16-]

   [16]*Including, but not limited to, corticosteroids exceeding 10 mg/day of prednisone equivalent, allergy injections, immunoglobulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids or other similar or toxic drugs during the preceding 12-month period prior to screening. Low dose topical and intranasal steroid preparations used for a discrete period of time are permitted* (Section 8.1).

4. Known allergy or intolerance to treatments for influenza (including but not limited to oseltamivir, baloxavir marboxil, acetaminophen).

5. Known allergy to two or more classes of antibiotics (*e.g.*, penicillins, cephalosporins, fluoroquinolones, or glycopeptides).

6. Known allergy to excipients in the challenge virus inoculum.

7. Receipt or planned receipt of any investigational drug/investigational vaccine/licensed vaccine within 30 days prior to the date of CHI.
8. Prior enrollment in any influenza virus challenge study.
9. Currently enrolled in any investigational study or intends to enroll in such a study within the ensuing study period.
10. Receipt of any influenza vaccine six months prior to challenge or plans to receive influenza vaccine within 60 days post-challenge.
11. History of a previous severe allergic reaction of any kind with generalized urticaria, angioedema, or anaphylaxis.
12. Receipt of blood or blood products during the six months prior to the planned date of challenge.
13. History of blood donation during the two months prior to the planned date of challenge and or plans to donate blood for the duration of the study.
14. Any condition, to include medical and psychiatric conditions, that in the opinion of the Investigator, might interfere with the safety of the subject and/or study objectives.
15. Known close contact with anyone known to have influenza 7 days prior to challenge.
16. Acute medical condition with new prescription medication use in the last 30 days.
17. Significant abnormality altering the anatomy of the nose/nasopharynx[17] clinically significant nasal deviation, or nasal/sinus surgery within 180 days prior to challenge.
    [17]*including significant nasal polyps.*
18. History in the last five years of chronic or frequent intermittent sinusitis.
19. Recent history (180 days) of epistaxis or anatomic or neurologic abnormality impairing the gag reflex or contributing to aspiration.

Table 6: Clinical Laboratory Normal Ranges

Hematology	Normal Range
WBC $x10^3/\mu L$	3.70 -11.00
Absolute Lymphocyte count $x10^3/\mu L$	$\geq$ 1.0
Hgb g/dL (Female)	$\geq$ 11.0
Hgb g/dL (Male)	$\geq$ 12.5
Platelets cell $x10^3/\mu L$ EDTA	163 – 375

Platelets K/cu mm Citrate	125 – 375
Chemistry	
ALT IU/L (Female)	≤ 43
ALT IU/L (Male)	≤ 60
Creatinine mg/dL	≤ 1.4

6.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

6.2.1 Discontinuation of the Study Product

Not applicable

6.2.2 Subject Replacement

Subjects who withdraw, are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product will not be replaced. Subjects who withdraw, are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced.

6.2.3 Study Termination

If the study is prematurely terminated by the Sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6.2.4 Withdrawal during the Inpatient Period

Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study intervention for any reason.

Subjects will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the inpatient unit before CHI. The investigator should be explicit regarding study follow-up (*e.g.*, safety follow-up) that might be carried out once the subject undergoes CHI but decides to leave inpatient unit before discharge criteria are met.

Subjects will be strongly discouraged from withdrawing from the study post-challenge. Any subjects who withdraws from the study post-challenge but prior to meeting the discharge criteria outlined will be asked to sign a document stating that they are aware of the potential risks of developing influenza complications, they are aware of the risks of transmitting influenza virus to high-risk populations (Appendix C), and that they will stay sequestered in their homes and avoid contact with others until they have two negative tests for influenza virus by qualitative RT-PCR.

The PI, co-investigator or study team will counsel the subject on infection control practices to minimize any community transmission. Subjects without two consecutive days of negative tests for influenza virus by qualitative RT-PCR will be given one dose of baloxavir marboxil at the time of premature withdrawal from the study. For the purposes of safety and serological testing, the subject will also be asked to come for all follow-up visits. If the subject leaves the inpatient unit prior to Day 8, whether or not they are continuing to shed virus, the subject will be encouraged to return for follow-up visits per outpatient schedule (i.e., Visits 9, 10, 11 and phone call at Visit 12). If the subject does not return for scheduled follow-up visits, extensive effort (i.e., at least three documented contact attempts via phone calls, e-mails, text message, or private social media communication, etc., as voluntarily provided by the subject and based on local IRB recommendations, made on at least 3 separate days and followed by a certified letter if unsuccessful) will be made to locate the subject and reiterate that follow-up visits are strongly encouraged for safety reasons. These efforts will be documented in the subjects' records. See the MOP for Subject Contact Information. The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

6.2.5 Withdrawal during the Follow-up Period

Subjects may withdraw voluntarily from participation in the study at any time. Subjects will be strongly discouraged from withdrawing from the study during the follow-up period. For the purposes of safety and serological testing, the subject will also be asked to come for all follow-up visits. The subject will be encouraged to return for follow-up visits per outpatient schedule (i.e., Visits 9, 10, 11 and phone call at Visit 12). The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study. As discussed in 5.2.6, if a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

6.2.6 Reasons for withdrawal might include, but are not limited to the following:

- Subject no longer meets eligibility criteria (prior to challenge)
- Subject meets individual halting criteria (Section 9.6.1)
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up
- Subject becomes pregnant, if applicable

- Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)
- If the subject consents, every attempt will be made to follow all adverse events (AEs) through resolution or stabilization.
- Clinical samples/data may be used for study analyses despite withdrawing early from the study.

7 STUDY PROCEDURES

7.1 Screening Period

7.1.1 Study Visit 00A (Day -30 to -3)

The screening sequence is designed to minimize subjects study visits and needle sticks, while identifying exclusionary criteria early and before more time-intensive and costly tests are performed and before the inpatient challenge stay begins. The screening may occur over more than one day and may encompass more than one visit.

- Subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the ICF. The ICF will be signed prior to performing any study procedure.
- Demographic information will be obtained by interview of subjects.
- Eligibility criteria will be reviewed with subjects.
- Complete medical history will be obtained.
- All concomitant medications taken within 90 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility. Medications reported in the eCRF are limited to those taken within 30 days prior to CHI.
- Documentation of receipt of 2018/2019 influenza vaccine
- Subject receipt of non-seasonal influenza vaccine, including those that are experimental, product type (inactivated or live attenuated), vaccine virus strains (e.g., A/H3N2v, A/H5, A/H7, A/H9, etc.) and approximate date of vaccination will be recorded on the appropriate DCF, if known.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature) will be obtained.
- Physical exam
- A serum pregnancy test will be performed on all female subject of childbearing potential and result must be negative.
- Height and weight will be collected.
- Blood for safety labs (approximately 15 mL), including HIV, hepatitis B, and hepatitis C testing, and testing for white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, alanine transaminase (ALT) and creatinine (Cr) will be sent to a central lab for evaluation and uploaded and entered into Advantage eClinical[SM]. These

values must be confirmed to meet the eligibility criteria as outlined in the Inclusion Criteria (Section 5.1.1) prior to admission to the inpatient unit.

- Urine toxicology test (amphetamines, cocaine, and opiates,) will be performed at the Central Lab.
- 12-lead ECG will be performed and confirmed to be within normal limits or not clinically significant as determined by the PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 prior to admission to the inpatient unit.
- CXR will be performed and confirmed to be within normal limits or not clinically significant as determined by the PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 prior to admission to the inpatient unit. Among female subjects of childbearing potential, a repeat urine pregnancy test will be performed locally if >7 days have passed since the negative serum pregnancy test was drawn. The result of the urine pregnancy test must be negative before the CXR is performed.
- Eligibility criteria will be reviewed with subjects in the week prior to admission to the inpatient unit to confirm no new exclusion criteria have been met since the review at initial enrollment.

7.2 Inpatient Period

7.2.1 Study Visit 00B (Day -2)

- The subjects will be admitted to inpatient unit.
- Eligibility criteria will be reviewed with subjects.
- Subject's willingness to participate will be reconfirmed and documented in the study records prior to performing any further study procedures. The subject will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the inpatient unit before virus is administered.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature) will be obtained.
- Weight will be collected for calculation of BMI (with height collected on Day -30 to -3).
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Medical history will be obtained.
- All concomitant medications will be reviewed with subjects for accuracy and completeness. Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility.

- A urine pregnancy test will be performed locally on all female subjects of childbearing potential and a positive result will exclude the subject from receipt of study product.
- Blood (approximately 20 mL) for antibody and cytokine assays
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (approximately 48 mL) for T and B immunology (cryopreserved PMBC)
- Blood (approximately 2.5 mL) for transcriptomics
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed to assure that no subject has a respiratory viral infection (positive results will exclude the subject from enrollment, and additional subjects may be admitted as replacements if any subjects are excluded prior to challenge)..
- NLF will be collected for baseline immunology assays.
- Urine toxicology test (amphetamines, cocaine, opiates, and cannabinoids) will be performed locally. Results must be negative for subject to continue participation in the study.

7.2.2 Study Visit 00C (Day -1)

- The subjects will remain in the inpatient unit.
- Eligibility criteria will be reviewed with subjects.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature) will be obtained.
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed to assure that no subject has a respiratory viral infection (positive results will exclude the subject from enrollment, and additional subjects may be admitted as replacements if any subjects are excluded prior to challenge). Quantitative RT-PCR and culture for influenza will be performed on the same NP swab specimen.
- NLF will be collected for baseline immunology assays **if not collected at Day -2**.

- Subjects will be trained on Flu-PRO Survey Instrument and Validation Diary.

7.2.3 Study Visit 01 (Day 1) Before Challenge

- Subjects will remain in the inpatient unit.
- Eligibility criteria will be reviewed with subjects.
- Informed consent will be reviewed with subjects.
- Baseline local and systemic symptoms will be assessed using daily Flu-PRO Survey Instrument and Validation Diary prior to challenge. All subjects will perform self-assessment using the Flu-PRO Survey Instrument Validation Diary daily during the inpatient stay to generate a symptom severity.
- Vital signs (including SpO_2*, HR, RR, and BP and oral temperature) will be obtained. (*For data analysis, this measurement of SpO_2 will be considered baseline.)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility.
- Medical history will be obtained.
- Blood (approximately 2.5 mL) for transcriptomics.
- Subjects will receive study influenza challenge virus. The challenge virus (A/Bethesda/MM2/H1N1) will be administered intranasally at a dose of 2 mL of approximately $5x10^6$ $TCID_{50}$. Intranasal challenge will be carried out using the MAD Nasal sprayer device (Wolfe-Tory Medical, Salt Lake City, Utah) attached to a 1 mL syringe. One mL of virus will be delivered in each nostril of a recumbent subject.

7.2.4 Study Visit 01 (Day 1) Post-challenge

- Subjects will remain in the inpatient unit.
- Post-challenge vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.

- All AEs and SAEs, will be recorded on the appropriate DCF.

7.2.5 Study Visit 02 (Day 2)

- Subjects will remain in the inpatient unit.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- All concomitant medication will be reviewed.
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- NLF will be collected for immunology assays.
- Blood for safety labs (approximately 10 mL), including white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), creatinine (Cr) will be sent to a central lab for evaluation and uploaded and entered into Advantage eClinical[SM].
- Blood (approximately 5 mL) for cytokine assays
- Blood (approximately 2.5 mL) for transcriptomics
- All AEs and SAEs, will be recorded on the appropriate DCF.

7.2.6 Study Visit 03 (Day 3)

- Subjects will remain in inpatient unit.
- All concomitant medication will be reviewed.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)

- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- All AEs and SAEs will be recorded on the appropriate DCF.

7.2.7 Study Visit 04 (Day 4)

- Subjects will remain in inpatient unit.
- All concomitant medication will be reviewed.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Blood for safety labs (approximately 10 mL), including white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), creatinine (Cr) will be sent to a central lab for evaluation and uploaded and entered into Advantage eClinical[SM].
- Blood (approximately 5 mL) for cytokine assays
- From at least a subset of participants: Blood (approximately 15 mL) for ASC (fresh PBMC)
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B cell phenotyping (cryopreserved PBMC)
- Blood (approximately 32 mL) for T and B cell immunology (cryopreserved PMBC)
- Blood (approximately 2.5 mL) for transcriptomics
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- NLF will be collected for immunology assays.

- All AEs and SAEs will be recorded on the appropriate DCF.

7.2.8 Study Visit 05 (Day 5)

- Subjects will remain in inpatient unit.
- All concomitant medication will be reviewed.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- All AEs and SAEs will be recorded on the appropriate DCF.

7.2.9 Study Visit 06 (Day 6)

- Subjects will remain in inpatient unit.
- All concomitant medication will be reviewed.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- NLF will be collected for immunology assays.
- Blood (approximately 5 mL) for cytokine assays

- From at least a subset of participants: Blood (approximately 15 mL) for ASC (fresh PBMC)
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (approximately 32 mL) for T and B immunology (cryopreserved PMBC)
- ECG will be performed.
- All AEs and SAEs will be recorded on the appropriate DCF.

7.2.10 Study Visit 07 (Day 7)

- Subjects will remain in inpatient unit.
- All concomitant medication will be reviewed.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM (see MOP for details)
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- All AEs and SAEs, will be recorded on the appropriate DCF.

7.2.11 Study Visit 08 (Day 8)

- Subjects begin the day in the inpatient unit and will remain if discharge criteria are not met.
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All concomitant medication will be reviewed.
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary. On this day, subjects may complete the self-assessment earlier than previous days if they are being discharged to home. New or changes in symptoms

between the time of self-assessment on this day and 5 PM (the usual time for self-assessment) will be captured in the Flu-PRO Survey Instrument and Validation Diary on Day 9 post-challenge (see MOP for details).

- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- NLF will be collected for immunology assays.
- Blood for safety labs (approximately 10 mL), including white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), creatinine (Cr) will be sent to a central lab for evaluation and uploaded and entered into Advantage eClinical^{SM}.
- Blood (approximately 20 mL) for antibody and cytokine assays
- From at least a subset of participants: Blood (approximately 15 mL) for ASC (fresh PBMC)
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (approximately 32 mL) for T and B immunology (cryopreserved PMBC)
- Blood (approximately 2.5 mL) for transcriptomics
- Subjects will be discharged once they meet all of the following discharge criteria (subjects meeting discharge criteria may leave after receipt of qualitative RT-PCR results for the day):
    - Have two consecutive qualitative RT-PCR tests on NP swabs (that are performed on Day 7 or thereafter on consecutive days) that are negative for influenza A using a multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®).
    - Are afebrile (< 100.4°F/38.0°C).
    - $SpO_2 \geq 95\%$ on room air
    - Show no moderate or severe influenza signs or symptoms by clinical evaluation.
    - Are clinically and hemodynamically stable for at least 48 hours (per the evaluation of the study physician).

- A single dose of baloxavir marboxil will be offered to all subjects that have not had two consecutive qualitative RT-PCR tests negative for influenza A.
- All AEs and SAEs, will be recorded on the appropriate DCF.

7.2.11.1 Unscheduled Inpatient Days After Day 8

- Subjects who are still shedding on Day 8 will remain in the inpatient unit and will continue to be tested until they have qualitative NP swabs that are negative for influenza A on two consecutive days
- Vital signs (including SpO_2, HR, RR, BP and oral temperature will be obtained approximately within each 8-hour period, or three times per day while subject is awake, and as clinically indicated).
- All concomitant medication will be reviewed.
- All subjects will perform self-assessment using the Flu-PRO Survey Instrument and Validation Diary once daily at approximately 5 PM through day 14 post challenge (see MOP for details).
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- A qualitative multiplex respiratory virus assay on NP swab specimen (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) will be performed.
- Subjects will be discharged once they meet all of the following discharge criteria (subjects meeting discharge criteria may leave after receipt of qualitative RT-PCR results for the day):
    - Have two consecutive qualitative RT-PCR tests on NP swabs (that are performed on Day 7 or thereafter on consecutive days) that are negative for influenza A using a multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®).
    - Are afebrile (< 100.4°F/38.0°C).
    - $SpO_2 \geq 95\%$ on room air
    - Show no moderate or severe influenza signs or symptoms by clinical evaluation.
    - Are clinically and hemodynamically stable for at least 48 hours (per the evaluation of the study physician).
    - All AEs and SAEs, will be recorded on the appropriate DCF.

- A single dose of baloxavir marboxil will be offered to all subjects that have not previously received a dose and have not had two consecutive qualitative RT-PCR tests negative for influenza A.

7.3 Follow-up Period

7.3.1.1 Post-discharge (Approximately Day 9 to Day 14)

- After discharge and through Day 14 post-challenge, subjects continue to perform daily self-assessment using the Flu-PRO Survey Instrument and Validation Diary at approximately 5 PM through day 14 post challenge.

7.3.1.2 Study Visit 09 (Day 15 ± 3 Days)3Days

- Subjects will visit the study clinic.
- Study personnel will collect the Flu-PRO Survey Instrument and Validation Diary from subjects including symptoms experienced from Days 9 to 14 post challenge.
- All concomitant medications will be reviewed.
- Vital signs including HR, RR, BP and oral temperature will be obtained.
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Blood (approximately 5 mL) for cytokine assays
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (approximately 40 mL) for T and B immunology (cryopreserved PMBC)
- Blood (approximately 2.5 mL) for transcriptomics
- NLF for immunology assays.
- All AEs and SAEs will be recorded on the appropriate DCF.

7.3.1.3 Study Visit 10 (Day 29 ± 3 Days)

- Subjects will visit the study clinic.
- All concomitant medication will be reviewed.
- Vital signs including HR, RR, BP and oral temperature will be obtained.

- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Blood (approximately 20 mL) for antibody assays
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (approximately 40mL) for T and B immunology (cryopreserved PMBC)
- NLF for immunology assays.
- All unsolicited AEs and SAEs will be recorded on the appropriate DCF.

7.3.1.4 Study Visit 11 (Day 61 ± 5 Days)

- Subjects will visit the study clinic.
- All concomitant medication will be reviewed.
- Vital signs including HR, RR, BP and oral temperature will be obtained.
- Targeted physical exam (lung, heart, oral/pharyngeal, and neck exams) will be performed; additional clinical evaluation will be performed if clinically indicated.
- Blood (approximately 20 mL) for antibody assays
- From at least a subset of participants: Blood (approximately 8 mL) for innate, T and B phenotyping (cryopreserved PBMC)
- Blood (40mL) for T and B immunology (cryopreserved PMBC)
- NLF for immunology assays.
- All SAEs will be recorded on the appropriate DCF.

7.3.2 Final Study Visit -- Telephone Follow-up (Day 91 ± 7 Days)

- A phone call for safety follow up will be performed.
- All SAEs will be recorded on the appropriate DCF.

7.4 Early Termination Visit

Any of the following activities may be performed at the Early Termination Visit on subjects who withdraw or are withdrawn or terminated from this study.

- All concomitant medications will be recorded on the appropriate DCF.

- Subject self-assessment using the Flu-PRO Survey Instrument and Validation Diary will be reviewed (if within 14 days post-challenge).
- Vital signs, including oral temperature, pulse, RR, and BP may be obtained.
- A targeted physical examination, may be performed
- Blood or nasal specimens for safety or research purposes may be collected depending on when this visit occurs.
- All AE/SAEs will be recorded on the appropriate DCF per Section 9.

7.5 Unscheduled Study Visits

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

- All concomitant medications will be recorded on the appropriate DCF.
- Subject self-assessment using the Flu-PRO Survey Instrument and Validation Diary will be reviewed (if within 14 days post-challenge).
- Vital signs, including oral temperature, pulse, RR, and BP may be obtained if indicated.
- A targeted physical examination, may be performed
- Blood or nasal specimens for safety or research purposes may be collected depending on when this visit occurs.
- All AE/SAEs will be recorded on the appropriate DCF.

7.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site PI, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the data coordinating center (DCC) protocol deviation reporting procedures.

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

8 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

8.1 Clinical Evaluations

Specifics of clinical and laboratory evaluations are in Table 11(Appendix A).

Screening for eligibility will occur from Days -30 to -3 to determine health status of potential subjects. This will include the following clinical evaluations:

- A complete medical and medication history
- Review of concomitant medications
- Review of receipt of 2018/2019 influenza vaccine
- Subject receipt of non-seasonal influenza vaccine, including those that are experimental, product type, vaccine virus strains and approximate date of vaccination (Section 7.1.1)
- Height and weight will be collected.
- Vital signs (including SpO_2, RR, HR, BP, oral temperature)
- Physical exam
- Collection of blood for screening and baseline safety laboratory testing
- Urine for toxicology
- ECG
- Serum pregnancy test
- Baseline PA and lateral CXR (Among women of childbearing potential, a repeat urine pregnancy test will be performed locally if >7 days have passed since the negative serum pregnancy test was drawn. The result of the urine pregnancy test must be negative before the CXR is performed.)

After enrollment, the subjects will be admitted to the inpatient unit (Day -2). Baseline assessments and testing will be performed after admission and the subjects will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the inpatient unit before challenge virus is administered. The following clinical evaluations will be performed in the 48 hours before challenge (Days -2 and Day -1):

- Vital signs (including SpO_2, RR, HR, BP, oral temperature)
- Targeted physical exam (including lung, heart, oral/pharyngeal, and neck exams)
- Weight will be collected.
- Complete medical and medication history
- Review of concomitant medications
- Urine for toxicology; results must be negative for subject to be eligible for challenge on day 1.
- A urine pregnancy test will be performed locally on all female subjects of childbearing potential and a positive result will exclude the subject from receipt of study product.

- NLF will be collected for immunology assays on Day -2. If not collected on Day-2, NLF may be collected on Day -1, but not both days.
- NP swab will be collected for testing for qualitative evaluation of viral respiratory infection

On Day 1, the challenge virus will be administered. Baseline evaluations before CHI will include the following:

- Local and systemic symptom assessment using Flu-PRO Survey Instrument and Validation Diary
- Targeted physical exam (including lung, heart, oral/pharyngeal, and neck exams)
- Complete medical and medication history
- Review of concomitant medications
- Vital signs including SpO_2, HR, RR, BP, oral temperature

Subjects will be followed for a minimum of 7 days post-challenge (through Study Day 8) in the inpatient unit. The following clinical evaluations will be performed during this post-challenge, inpatient period:

- Local and systemic symptom assessment using Flu-PRO Survey Instrument and Validation Diary
- Targeted physical exam including height, weight, clinical evaluation (lung, heart, oral/pharyngeal, and neck exams)
- Complete medical and medication history
- Review of concomitant medications
- Vital signs including SpO_2, HR, RR, BP, oral temperature
- ECG will be performed
- NP Swab will be collected for testing for qualitative evaluation of viral respiratory infection
- Collection of blood for clinical laboratory tests

8.1.1 Research Procedures

- Flu-PRO Survey Instrument and Validation Diary to generate a symptom severity score
- Collection of blood for antibody and cytokine assays
- Collection of blood for transcriptomics
- From at least a subset of participants: Collection of blood for ASC (fresh PBMC)
- From at least a subset of participants: Collection of blood for innate, T and B phenotyping (cryopreserved PBMC)
- Collection of blood for T and B immunology (cryopreserved PMBC)
- Collection of NLF for immunology assays
- Collection of NP swab for viral shedding and viral load assessment.

8.1.2 Assessment of Concomitant Medications/Treatments other than Study Product

Concomitant medications will be reviewed at every study visit through the end of the study. Women of child bearing potential in a heterosexual relationship must agree to use true abstinence or use at least one acceptable primary form of contraception through the end of the study.

Receipt of any influenza vaccine during the 2018/2019 influenza vaccine season regardless of the date of receipt) will be documented. Subject receipt of non-seasonal influenza vaccine, including those that are experimental, product type, vaccine virus strains and approximate date of vaccination (Section 7.1.1)

The following prescription or over-the-counter medications cannot be used within 7 days prior to admission to the inpatient unit and through the inpatient stay, unless approved by the investigator: oseltamivir, zanamivir, peramivir, baloxavir marboxil, amantadine (generic) and rimantadine (Flumadine and generic), aspirin, intranasal steroids, decongestants, antihistamines, and other non-steroidal anti-inflammatory drugs (NSAIDs).

Subjects who use asthma medications including inhaled, oral, or IV corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics, will be excluded from the study.

Any medications that may be associated with impaired immune responsiveness including, but not limited to, corticosteroids exceeding 10 mg/day of prednisone equivalent, allergy injections, immunoglobulin, interferon, immunomodulators, cytotoxic drugs, or systemic corticosteroids or other similar or toxic drugs cannot be used during the preceding 12-month period prior to screening. Low dose topical and intranasal steroid preparations used for a discrete period of time are permitted.

Subjects should not have received any investigational drug/investigational vaccine/licensed vaccine within 30 days prior to the planned date of CHI.

Subjects should not have received influenza vaccine six months prior to challenge and must not plan to receive an influenza vaccine 60 days post challenge.

Subjects should not have received blood or blood products during the six months prior to the planned date of CHI.

8.1.3 Assessment of Subject Compliance with Study Intervention/Investigational Product/Investigational Device

Subjects will be directly observed at the time of dosing by a member of the clinical research team who is licensed to administer the study product. Administration will be documented and entered into the eCRF.

8.1.4 Non-Research Standard of Care

Baloxavir marboxil (Xofluza)may be given to each subject who has not had two consecutive days of negative qualitative RT-PCR tests for influenza virus on Study Day 8. Baloxavir marboxil is a polymerase acidic endonuclease inhibitor indicated for the treatment of acute uncomplicated influenza in patients 12 years of age and older [40]. Baloxavir marboxil is licensed as a single-dose drug.

8.1.5 Non-Research Standard of Care

Subjects who continue to shed virus beyond Day 8 will undergo routine daily evaluations as described in Sections 6 and 7. If the subject is considered by the study physician to be clinically well, no additional changes to study procedures will be made. The study physician may treat the subject with symptom-directed, over-the-counter therapies as permitted by the study protocol. If the subject meets criteria for escalation of care, evaluation and treatment will be at the discretion of the consulting physician.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

- Hematology at Screening: white blood cells (WBCs), absolute lymphocyte count, hemoglobin, and platelets (if platelets in the EDTA tube are clumped and the result is not available, platelets may be repeated in a citrate tube)
- Hematology at Follow-up (at Days 2, 4, and 8): white blood cells (WBCs), absolute lymphocyte count, hemoglobin, and platelets (if platelets in the EDTA tube are clumped and the result is not available, platelets may be repeated in a citrate tube)
- Biochemistry at Screening: alanine transaminase (ALT) and creatinine (Cr)
- Biochemistry at Follow-up (at Days 2, 4, and 8): alanine transaminase (ALT) and creatinine (Cr)
- Hepatitis B and Hepatitis C, HIV at Screening.
- A urine toxicology test (amphetamines, cocaine, and opiates,) at screening (to be performed at the central laboratory) and on the day of admission to the inpatient unit (amphetamines, cocaine, opiates, and cannabinoids) (to be performed locally).
- Serum HCG pregnancy test (for female subjects of childbearing potential) at screening to be performed at the Central Laboratory
- Urine HCG pregnancy test (for female subjects of childbearing potential) to be done before the baseline CXR (if > 7 days have passed since the negative serum pregnancy test was drawn) and on the day of admission to the inpatient unit, to be performed locally.

- Qualitative multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) on NP swab

8.2.2 Research Assays

- HAI, MN, NAI, anti-HA-stalk antibody titers from serum
- sIgA titers from NLF
- Human leukocyte antigen (HLA) class I and II alleles from blood
- Systemic cytokines and chemokines from serum
- Mucosal cytokines and chemokines from NLF
- Transcriptional responses from whole blood
- Plasmablasts (ASC) in circulation (from PBMC)
- Influenza-specific B and T cell immune responses from PBMCs
- Immunophenotyping of innate, B and T cell subsets in NLF
- Quantitative RT-PCR for influenza from NP swab specimen.

8.2.2.1 Laboratory Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

8.2.2.2 Laboratory Specimen Shipping

Specimen shipment will occur at intervals during the course of this study following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

Specimens for clinical screening and safety laboratory evaluations will be transported from the participating sites to the central (clinical) laboratory. Urine toxicology on the day of admission will be performed at the local laboratory.

Specimens for HAI, MN, NAI and anti-HA-stalk antibody assays, transcriptomics and Plasma/PBMC-based and NLF-based assays will be shipped from the participating sites to DMID Clinical Materials Services (CMS) and then provided by the CMS to the appropriate laboratory in a blinded manner.

Further instructions for specimen shipment are included in the central (clinical) laboratory manual and protocol-specific MOP, as appropriate.

9 ASSESSMENT OF SAFETY

9.1 Assessing and Recording Safety Parameters

Signs, symptoms, laboratory findings that are part of MMID from the time of CHI through 14 days post-challenge will not be considered adverse events. These signs, symptoms and laboratory findings (mild and moderate) which define MMID include the following: arthralgia, chest tightness, chills, conjunctivitis, nasal congestion, sinus congestion, coryza, decreased appetite, diarrhea, cough, dyspnea/shortness of breath, fatigue/tiredness, fever (>38.0°C), headache, lymphopenia (<1000 cells/mL), myalgia, nausea, oxygen saturation decrease by ≥3% from baseline, rhinorrhea, sore throat, and sweats.

Safety will be assessed by the frequency and severity of:

1. Study product-related serious adverse events occurring from the time of the challenge through the end of the study (approximately 3 months post-challenge).

2. Clinical safety laboratory adverse events occurring from the time of CHI through study Day 8. Parameters to be evaluated include: white blood cells (WBCs), absolute lymphocyte count (except as part of MMID), hemoglobin, platelets, and alanine transaminase (ALT), and creatinine (Cr).

3. Adverse Events – non-serious adverse events occurring from the time of CHI through approximately 30 days post-challenge. See below for how adverse events are defined.

9.1.1 Adverse Events (AEs)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

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 (investigational) 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.

In this study, solicited symptoms of MMID will be collected from the time of CHI through 14 days post-challenge and will **not** be considered adverse events. Any influenza signs, symptoms or lab findings determined by the clinician to have exceeded the expected severity of MMID will

be considered unsolicited adverse events and captured on the appropriate data collection form and electronic case report form (eCRF). Events that are not consistent with illness due to influenza will be considered unsolicited AEs as well. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the study will be documented appropriately regardless of relationship. AEs will be followed through resolution. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any chronic or stable medical condition (Section 6.1) that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

9.1.1.1 Adverse Events Grading

AEs must be assessed for severity and relationship to study product (see definitions below). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate DCF and entered into the eCRF.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site PI or sub-investigator using a protocol-defined grading system (described below in this Section). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product: The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a CHI, the study product must always be suspect. To help assess, the following guidelines are used:

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

Adverse Events Grading: All AEs and laboratory findings (that are not part of MMID) will be graded for severity and assessed for relationship to study product (see definitions). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. 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.

Oral temperature will be graded as follows:

Table 7: Severity Grading for Oral Temperature

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral†	38.1°C – 38.4°C 100.6°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F
*A fever can be considered not related to the study product if an alternative etiology can be documented. †Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature			

Pulse, blood pressure, respiratory rate, and SpO_2 will be graded as follows:

Table 8: Pulse, BP, RR and SpO_2 Adverse Event Grading

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia – beats per minute	45 – 46	40 – 44	<40
Tachycardia – beats per minute	100 – 130	131 – 155	>155
Hypotension (systolic) mmHg	80 – 84	75 – 79	<75
Hypotension (diastolic) mmHg	50 – 54	45 – 49	<45

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hypertension (systolic) mmHg	140 – 155	156 – 160	>160
Hypertension (diastolic) mmHg	90 – 100	101 – 110	>110
SpO_2 (%)	92-94	89-91	<89
RR (increase) (bpm)	21-24	25-29	>30
RR (decrease) (bpm)	9-11	6-8	<6

Clinical safety laboratory values# will be graded as follows:

Table 9: Clinical Safety and Laboratory AEs Grading

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC $x10^3/\mu L$ (Decrease)	2.50 – 3.69	1.50 – 2.49	<1.50
WBC $x10^3/\mu L$ (Increase)	11.01 – 15.09	15.10 – 20.00	>20.00
Absolute Lymphocyte count x $10^3/\mu L$ (Decrease)	0.75 - 0.99	0.50 - 0.74	< 0.50
Hgb g/dL (Decrease) (Female)	10.1 – 10.9	8.5 – 10.0	<8.5
Hgb g/dL (Decrease) (Male)	11.0 – 12.4	9.5 – 10.9	<9.5
Platelets cell x $10^3/\mu L$ (Decrease) EDTA	140 – 162	100 – 139	<100
Platelets x $10^3/\mu L$ (Increase) EDTA	376 – 550	551– 750	>750
Platelets K/cu mm (Decrease) Citrate	115-124	100-114	<100
Platelets K/cu mm (Increase) Citrate	376 - 550	551-750	>750
Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
ALT IU/L (Increase) (Female)	44 – 100	101 – 200	>200
ALT IU/L (Increase) (Male)	61- 138	139 – 275	>275

Creatinine mg/dL (Increase)	1.5 – 1.8	1.9 – 2.2	> 2.2

9.1.2 Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered a SAE if, in the view of either the site principal investigator or Sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event[1],
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an ER or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

[1] Life-threatening adverse event. An AE is considered "life-threatening" if, in the view of either the site principal investigator or Sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician (for investigational new drug application [IND] studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator).
- Reviewed and evaluated by DMID, an Independent Safety Monitor (ISM) (as deemed necessary), the Safety Monitoring Committee (SMC) (periodic review unless related), and the IRB/IEC.

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

The site PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 is responsible for recording all AE/SAEs that are observed or reported during this study, regardless of relationship to study product. AE/SAEs, abnormal laboratory test values or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately, using a local laboratory as necessary. In determining eligibility, refer to 6.1and the protocol-specific MOP.

9.3 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

Solicited Symptoms: Influenza symptoms elicited by the Flu-PRO Survey Instrument and Validation Diary (Appendix D).

Unsolicited Events: Unsolicited events are any non-serious adverse events occurring from the time of CHI through approximately 30 days post-challenge.

9.4 Reporting Procedures

9.4.1 Reporting Serious Adverse Events

Any AE that meets a protocol-defined serious criterion must 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 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM (as deemed necessary) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572 will report the event to the DMID Pharmacovigilance Group.

9.4.2 Regulatory Reporting for Studies Conducted Under DMID Sponsored- IND

Following notification from the site PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 15721572, DMID, as the IND Sponsor, will report any suspected unexpected SAE. DMID will report an AE as a suspected unexpected AE only if there is evidence to suggest a causal relationship between the study intervention and the AE. DMID will submit an IND safety report to the FDA and will notify all participating site PIs (*i.e.*, all PIs to whom the Sponsor is providing drug under its IND(s) or under any PI's IND(s) of potential serious risks from clinical studies or any other source, as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than seven calendar days after the Sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the Sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.4.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage electronic data capture system (Advantage eClinical) on the Pregnancy Report form. No further study product will be administered to pregnant subjects, but with the subject's permission a venous blood sample for serological assays will be collected per protocol, however large volume blood samples for cellular immunological assays will be discontinued, and the subject will continue to be followed

for safety for the duration of this study. Efforts will be made to follow all pregnancies reported during the course of this study to pregnancy outcome pending the subject's permission.

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

AEs will be assessed (per Section 9.1.1) and followed from initial recognition of the AE through end of the protocol defined follow-up period.

SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined the follow-up period.

Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

9.6 Halting Rules

9.6.1 Study Halting Criteria

Additional enrollment and study interventions/administration of study products in this study will be halted for Safety Monitoring Committee (SMC) review/recommendation if any of the following are reported:

1. Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within one day after challenge.
2. Four or more subjects (approximately ≥5% of the anticipated sample size) experience the same related grade 3 AE of any kind after challenge through the inpatient unit period and related to study product.
3. Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within three days after challenge.
4. Any subject experiences a related SAE during the inpatient unit period.
5. Any subject develops severe influenza (Section 2.3.2) during the inpatient unit period and requires admission to an Intensive Care Unit (ICU), and/or receipt of ICU level of care.
6. Any medically significant safety issue that the PI determines should halt the study.

9.7 Safety Oversight

9.7.1 Safety Monitoring Committee (SMC)

This clinical study will utilize an SMC, which is an independent group of experts that advises the DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate expertise to contribute to the interpretation of the data from this study. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the

scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

As defined in the charter, the SMC will review data at specified times during the course of the study for subject and overall study progress and will conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study. The SMC will hold the following meetings; an organizational meeting prior to subject enrollment, any ad hoc meetings when a halting rule is met or due to specific safety issues as the SMC deems necessary, and a final meeting to review cumulative safety data.

10 HUMAN SUBJECTS PROTECTION

10.1 Institutional Review Board/Independent Ethics Committee

Each institution engaged in this research will hold a current Federal Wide Assurance (FWA) issued by the Office for Human Research Protections (OHRP) for federally funded research. The IRB must be registered with OHRP [OHRP-only or OHRP/FDA] as applicable to the research. The IRB FWA number will be provided to DMID.

Each site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s) and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

10.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. During recruitment, investigator or designee may obtain information regarding eligibility through oral or written communication with the prospective subject. The screening may occur by phone using an IRB approved process that ensures confidentiality. The information obtained from this phone call assessment will remain locally at the site and will not be entered into the Advantage EDC data base unless the subject is enrolled.

At the first screening visit before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the study, verbally and with a written consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study,

risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject. The subject will be asked to consent specifically to genetic testing (HLA testing and transcriptomics) planned for this study.

Subjects will also receive an explanation that the study involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the study that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the study, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the study. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the study, as well as any anticipated prorated payments, if any, to the subject for participating in the study. They will be informed of whom to contact (*e.g.*, the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the study may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of study procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the study are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research study and have the opportunity to discuss this study with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved, and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this study.

Once signed, a copy of the informed consent form will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the study. The rights and welfare of the subject(s) 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.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this study in accordance with IRB requirements. The informed consent document will be updated, and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

10.3 Consent for Future Use of Stored Specimens and Data

Subjects who agree to participate in this study will have venous blood collected for clinical safety laboratory evaluations, serological and cellular immunology assays, as well as for transcriptomic profiling assessments. The amount of venous blood collected for these assays may exceed the amount required to perform per protocol (PP) defined assays, and this excess/residual blood and corresponding serum, plasma and PBMCs will be designated for future research use and stored indefinitely at a central storage facility. Similarly, subjects will also have NP swabs and NLF collected for clinical laboratory evaluations and immunology assays. The NP swab supernatant can be stored after it is used to test for respiratory viruses. The NLF collected for immunology assays exceeds the amount required to perform per protocol (PP) defined assays. The NP swabs and NLF excess/residual fluid and corresponding isolated cells will be designated for future research use and stored indefinitely at a central storage facility.

Samples designated for future research use may be used to determine additional immunological assessments that may include but are not limited to antibody epitope mapping, B and T cell repertoire determination, nontraditional immune assay development, determination of innate immune factors and the ability of challenge virus-induced antibodies to cross-react to different influenza proteins and virus strains. These blood samples might be used in new or different immunological laboratory tests, to provide information for the development of new vaccines, or for the studies of influenza or other infections. Subjects will be asked to consent specifically to future genetic testing, including whole genome sequencing An IRB will review future research

prior to the use of identifiable specimens or data. Future research use specimens, upon written request and approval from DMID, may be shared with investigators at the participating VTEU sites, with other investigators or company designated research laboratories for purposes of conducting additional immunological assessments other than PP analysis. DMID would authorize shipment from the DMID CMS. There are no benefits to subjects in the collection, storage and future research use of their samples/specimens. Future research use samples/specimens will not be sold or used directly for production of any commercial product. Each sample/specimen will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. Reports from future research studies performed using subjects' samples/specimens will NOT be kept in their health records.

Subjects will be asked to consent to the future research use of excess/residual specimens at the time of enrollment. Subjects will be asked to consent to sharing genetic information and samples. Subjects may withdraw permission to use samples for future use at any time. They will need to contact the study site and the samples will be removed from the study repository after this study is completed and documentation will be completed that outlines the reason for withdrawal of permission for future use of samples.

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

This study will be inclusive of all healthy adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background.

10.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the Sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the Sponsor or governmental regulatory agencies 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 subjects in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

10.6 Certificate of Confidentiality

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate 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 for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a subject in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, or for the purposes of other research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

10.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this study. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. Subjects may be compensated for their participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this study, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this study.

11 STATISTICAL CONSIDERATIONS

11.1 Introduction

The goal of this study is to assess the impact of pre-existing immunity to A/California/04/2009/H1N1 Variant Influenza A virus on clinical responses and immune responses after subjects are challenged intranasally with 2 mL of approximately $5x10^6$ tissue culture infective dose ($TCID_{50}$) of the virus. The study will evaluate and further refine the H1N1 CHI model across multiple sites, which will help develop the model for future vaccine studies.

11.2 Study Hypotheses

This study is not designed to achieve pre-set levels of power and precision to address the primary, secondary, or exploratory objectives. The power and precision allowed by the study's sample size are discussed in Section 11.3, and the general methodology planned to address study objectives is described in Section 11.5. Specific null and alternative hypotheses and details about planned tables, figures, and data listings will be defined in a separate statistical analysis plan.

11.3 Sample Size Considerations

11.3.1 Study Design

This is a CHI study of A/Bethesda/MM2/H1N1 virus infection to assess clinical response, immunological response, and safety. A total of up to 80 participants will receive controlled A/Bethesda/MM2/H1N1 virus inoculation.

Influenza A/Bethesda/MM2/H1N1 challenge will be performed using a 2 mL dose of approximately $5x10^6$ $TCID_{50}$/mL. The primary objective of the study will be to evaluate the association of symptomatic RT-PCR-positive influenza virus infection post-challenge and pre-existing HAI antibody titers in healthy subjects.

Details of the study design are presented in Section 4, and the schedule of events is given in Appendix A (Table 11).

11.3.2 Sample Size

The study is planned to enroll up to 80 subjects, all of whom will receive the study influenza challenge virus. While the study is not designed to test any specific null hypothesis or reach a pre-determined level of estimation precision, the following gives the precision and power for estimates and hypothesis tests of interest, respectively, based on this proposed sample size. Adjustments to p-values in the presence of multiple comparisons may be made, depending on the objective, but the calculations below do not account for this possibility. Details on adjustment for multiple comparisons will be described in the statistical analysis plan.

The primary objective is to evaluate the association between pre-existing (baseline) HAI antibody titers and MMID post-challenge. Table 9 below gives the power to detect varying decreases in the odds of a binary endpoint such as MMID with a one standard deviation (SD) increase in a continuous covariate (assumed to be normally distributed) among all subjects (n=80), via a Wald test from a logistic regression model at the α=0.05-level. This would roughly correspond to testing for an association between log-transformed baseline HAI titer and MMID.

Table 10: Power to detect odds ratios (OR) for a one SD increase in a continuous, normally distributed covariate in a logistic regression model at the α=0.05-level

	Power (%)			
Prevalence*	**OR=0.4**	**OR=0.5**	**OR=0.6**	**OR=0.7**
0.5	96.1	82.8	59.1	34.2
0.6	95.5	81.3	57.3	32.9
0.7	94.9	79.6	55.1	31.3

*The probability of a binary outcome such as MMID within the study population

Table 10 indicates the probability of observing one or more safety events, such as a solicited symptom or an SAE of a particular classification in all subjects (n=80), given underlying event probabilities.

Table 11: Probability (%) of observing at least one safety event, given varying underlying event probabilities and group sizes

True Event Probability	All Subjects (N=80)
10% (very common)	>99.9
1% (common)	55.2
0.1% (uncommon)	7.7
0.01% (rare)	0.8

Binomial confidence intervals (CI) are widest (have the least precision) when the response rate is 50%. With this event rate among all subjects (40 events among n=80 subjects), the corresponding exact Clopper-Pearson CI would be (38.6%, 61.4%).

11.4 Planned Interim Analyses

Interim analyses would only be used to terminate this trial in the event that unanticipated safety events were determined to be of sufficient concern to require such action by the sponsor. These assessments will not be made on the basis of testing a formal statistical hypothesis. As described in Section 9.7, an SMC will be convened by DMID to review participant, clinical, and safety data.

11.4.1 Interim Safety Review

An interim safety review may include enrollment and demographic information, clinical laboratory tests, reported influenza symptoms, and AE/SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC will meet and review these data at scheduled time points or ad hoc as needed during this trial, as delineated in the SMC charter. As an outcome of each review, the SMC will make a recommendation to continue, modify, or terminate this study. The trial will also be monitored to determine if any of the halting rules in Section 11.4.1are met, and if so, the SMC will be convened to review.

11.5 Final Analysis Plan

Clinical, safety, and immunology data through the last follow-up at Day 91 will comprise the primary clinical database for this study. Once the last subject has completed the final telephone follow-up, the primary clinical database will be cleaned, monitored, and locked. Analyses of safety, clinical, and available immunological data will be performed by the SDCC after the primary clinical database is locked and the detailed statistical analysis plan is finalized.

The CSR will be completed when all primary and secondary safety, clinical, and immunological endpoint data are available. Any available data from the exploratory endpoints may also be included. Additional exploratory endpoint data may be included in an addendum to the CSR, publication of manuscript(s), or other report.

A formal statistical analysis plan (SAP) that elaborates on the analyses indicated here will be developed and finalized prior to performing the final analysis.

11.5.1 Analysis Populations

The Safety Analysis (SA) population will include all subjects who received the study influenza challenge.

The modified Intent-to-Treat (mITT) population will consist of all subjects who received the study influenza challenge and were followed for the entire challenge period of the study.

The Per-Protocol (PP) population will include all subjects in the mITT population with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline
- Data from any visit that occurs substantially out of window
- Data from all visits subsequent to:
    - Major protocol deviations
    - Receipt of immunosuppression or any medications that may be associated with impaired responsiveness
    - Receipt of any investigational drug/investigational vaccine/licensed vaccine

- Receipt of blood or blood products, or blood donation

The Infected (INF) population will include only the infected subjects (i.e. those with detectable influenza virus) from the PP population.

The Symptomatic (SYMP) population will include only the subjects from the PP population who report symptoms throughout the challenge period.

11.5.2 Safety and Clinical Data

Summaries of safety and clinical data will be presented for the SA population. Analysis involving endpoints calculated over the course of the entire challenge period (e.g. MMID) will be done within the mITT population, or the PP, INF, or SYMP populations as appropriate.

Binary safety and clinical outcomes such as influenza infection, MMID, solicited symptoms, and SAEs will be presented as frequencies and percentages. Results will be presented overall and by infection status, and separately by study day where applicable. Associated confidence intervals and comparisons between infected and uninfected subjects via exact binomial tests will also be included, as appropriate.

Viral shedding will be summarized over the challenge period with the following: a binary yes/no variable separately by inpatient study day (Day 1 through Day 8) and over the entire challenge period; peak (quantitative) shedding and total shedding, as measured by the area under the curve (AUC) over the challenge period; the total number of days of shedding.

Solicited symptoms of influenza recorded via the Flu-PRO survey instrument will be summarized by extent (not at all, a little bit, somewhat, quite a bit, very much) for each study day, the maximum extent over the first 8 days, and the maximum extent over the entire study. Symptoms will also be analyzed by treating the extent as an ordinal variable (0=not at all, 1=a little bit, etc.) and calculating the mean scores in each domain (e.g. nose, throat, etc.) and across all collected symptoms. Symptoms will also be analyzed by taking the most extensive response over the follow-up period and dichotomizing into a binary variable (not at all versus at least a little bit) and using standard techniques, such as exact confidence intervals, to summarize the proportion of subjects reporting each symptom. Analysis of reported symptoms will be presented across all subjects and by infection status (RT-PCR-confirmed symptomatic, RT-PCR-confirmed asymptomatic, RT-PCR-negative symptomatic, RT-PCR-negative asymptomatic), and subjects may be compared by infection status using appropriate hypothesis tests. The Flu-PRO Survey Instrument will be assessed for its accuracy in predicting infection status in an exploratory analysis, by randomly splitting the data into training and testing data.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA) for preferred term (PT) and system organ class (SOC). SAEs will be reported by detailed listings showing the event description, MedDRA preferred term and SOC, relevant dates (challenge dates and AE onset dates), severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one

event in each MedDRA SOC and PT, cross tabulated by severity and relationship to the influenza challenge. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA categories will be computed. SAEs will be described by visit, over the challenge period and over the entire study.

Clinical laboratory data will be summarized by severity for each visit and as the maximum over all visits. Graphical presentations may include box plots and plots of changes from pre-infection measurements.

11.5.3 Immunological Data

Analysis of the primary objective will be done in both the mITT and PP populations, the latter as a sensitivity analysis. Analysis of secondary and exploratory immunological objectives will be done in the PP, INF, and/or SYMP populations, as appropriate.

The associations between MMID and variable baseline HAI titers (the primary analysis) will be assessed in the PP population via logistic regression, treating baseline titer as a continuous variable after appropriate transformation. Associations between other infection-related endpoints (secondary outcomes: asymptomatic infection; symptoms without detected influenza virus) and baseline HAI, NAI, or MN antibody titers will be assessed similarly. For asymptomatic infection, the analysis will be performed in the INF population to evaluate the likelihood of symptoms given influenza infection; for symptoms without detected influenza virus, the analysis will be performed in the SYMP population to evaluate the likelihood of infection given reported symptoms.

Study strain-specific immune responses will be summarized at each relevant study visit, overall and by infection status. Analyses for antibody titers will include the number and proportion of subjects with a titer ≥1:40, number and proportion of subjects achieving seroconversion (defined as either a pre-challenge titer <1:10 and a post-challenge titer ≥1:40 or a pre-challenge titer ≥1:10 and a minimum four-fold rise in post-challenge antibody titer), and Geometric Mean Titers (GMTs) along with corresponding 95% confidence intervals. Descriptive summary statistics will be provided for all assays and time points. The correlation between HAI, MN, and NAI antibody titers will be evaluated. Plots such as reverse cumulative distributions or longitudinal presentations of GMTs will be presented.

Exploratory objectives also include evaluating the associations between MMID and baseline HAI titers, as well as potential confounders age, sex, and receipt of seasonal influenza vaccine in the previous year. This will be assessed via multivariable logistic regression in the PP population.

Assays measured on continuous scales will be summarized by the mean, median, standard deviation, and range.

11.5.1 Transcriptomic Data

For the transcriptomics exploratory analysis, sequencing data will be pre-processed by removing adapters and low-quality reads and mapping sequences to the latest human reference genome using splice-aware alignment software such as *HISAT2* [44]. Gene expression quantification will be carried out by using the *Subread* software [45] using the latest Ensembl [46] gene model annotations as a reference. Systematic differences in sequence coverage between samples will be accounted for using the TMM method [47] as implemented in the *edgeR* [48] R package. Principal component analysis, non-metric multidimensional scaling, and hierarchical clustering analysis will be used to identify potential global outliers and systematic batch effect. Negative binomial models as implemented in *edgeR* [48] will be used to identify genes for each post-challenge day that were differentially expressed (DE) compared to pre-challenge. To control for testing multiple genes, the false-discovery rate (FDR) based on the Benjamini-Hochberg procedure [49] as implemented in the *p.adjust* R function will be applied. Genes with a FDR-adjusted p-value < 0.05 will be deemed to be significantly DE. The *pvclust* [50]R package will be applied to identify co-expressed DE gene clusters and cluster time trends will be visualized. Pathway enrichment analysis based on the latest MSigDB [51] and KEGG [52]databases as well as Blood Transcription Modules [53] accounting for gene length bias will be carried out using the implementation provided by the *goseq* [54] R package. Pathway maps color-coded by challenge effect will be provided for significantly enriched KEGG pathways. In addition, for each enriched pathway, time trends of the median of the average pathway response will be visualized. Additional details/analyses will be described in the SAP.

11.5.2 Genomics Data

To assess the association of subject HLA class I and II alleles with development of MMID post-challenge through Day 8, pre-existing immune status, and magnitude and breadth of the elicited immune responses post-challenge, regularized logistic regression (for binary outcomes) and regularized linear regression (for continuous outcomes) using HLA alleles as predictors as implemented in the *glmnet* [55] R packages will be utilized. Cross-validation will be used to select optimal regularization parameters. Additional details/analyses will be described in the SAP.

11.5.3 Missing Values and Outliers

All attempts will be made to collect all data per protocol. If all Flu-PRO Survey Instrument data for one study day are missing, the missingness will be indicated in related summaries, but no imputation is planned for endpoints derived from these data (e.g. MMID). No imputation for missing immunological or transcriptomic/genomic data is planned either.

Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each 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 and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aids or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the study.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site(s) and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and current documentations are maintained on site.

The DCC 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 participating site(s) for clarification and resolution.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Management Responsibilities

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue permanent ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the eCRF will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

The Sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

14.2 Data Coordinating Center/Biostatistician Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

14.3 Data Capture Methods

Clinical information (including, but not limited to, AE/SAEs, concomitant medications, receipt of 2018/2019 influenza vaccine, receipt of non-seasonal influenza vaccine, including those that are experimental, product type, vaccine virus strains and approximate date of vaccination (Section 7.1.1) will be recorded on the appropriate DCF, medical history, physical assessments, and clinical laboratory values) will be collected on data collection forms by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

14.4 Types of Data

Data for this study will include clinical, safety, and outcome measures (*e.g.*, clinical laboratory values, and immunological data).

14.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for 2 years after a marketing application is approved for the study product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the study product, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for future use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the site principal investigator when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

15 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet Sponsor, ICH/GCP guidelines and applicable regulations, and that this study is conducted in accordance with the protocol, protocol-specific MOP and applicable Sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will 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, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and will document site visit findings and discussions.

16 PUBLICATION POLICY

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this study the responsible party is NIAID/DMID which will register the study and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

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18 APPENDICES

Appendix A. Schedule of Events

Table 12: Schedule of Events

Evaluation	Enrollment/Screening Study Visit 00A Day -30 to -3	Inpatient Study Visit 00B Day -2	Inpatient Study Visit 00C Day -1	Inpatient Study Visit 1 Day 1	Inpatient Study Visit 2 Day 2	Inpatient Study Visit 3 Day 3	Inpatient Study Visit 4 Day 4	Inpatient Study Visit 5 Day 5	Inpatient Study Visit 6 Day 6	Inpatient Study Visit 7 Day 7	Inpatient Study Visit 8 Day 8	Follow-up Study Visit 9 Day 15±3	Follow-up Study Visit 10 Day 29±3	Follow-up Study Visit 11 Day 61±5	Final Study Visit 12 Day 91 ± 7	Early Termination Visit	Unscheduled Visit
Visit type[1]	c	i	i	i	i	i	i	i	i	i	i	c	c	c	p	c	c
Screening for eligibility	x																
Signed informed consent	x																
Review/confirm eligibility criteria	x[2]	x	x	x													
Review/confirm informed consent		x[3]		x													
Inpatient period		x[4]	x	x	x	x	x	x	x	x	x						
Discharge from inpatient											x[5]						
Treatment with antiviral											x[6]						
Demographics	x																
Medical History	x	x		x													
Height measurements	x[7]																
Weight measurements	x[7]	x[7]															
Concomitant medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	X
Physical Exam	x																
Targeted physical exam[8]		x	x	x	x	x	x	x	x	x	x	x	x	x		x[22]	x[22]

Evaluation	**Enrollment/Screening Study Visit 00A Day -30 to -3**	**Inpatient Study Visit 00B Day -2**	**Inpatient Study Visit 00C Day -1**	**Inpatient Study Visit 1 Day 1**	**Inpatient Study Visit 2 Day 2**	**Inpatient Study Visit 3 Day 3**	**Inpatient Study Visit 4 Day 4**	**Inpatient Study Visit 5 Day 5**	**Inpatient Study Visit 6 Day 6**	**Inpatient Study Visit 7 Day 7**	**Inpatient Study Visit 8 Day 8**	**Follow-up Study Visit 9 Day 15±3**	**Follow-up Study Visit 10 Day 29±3**	**Follow-up Study Visit 11 Day 61±5**	**Final Study Visit 12 Day 91 ± 7**	**Early Termination Visit**	**Unscheduled Visit**
Vital signs[9]	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x[22]	x[22]
SpO_2[10]	x	x	x	x	x	x	x	x	x	x	x						
Influenza Challenge				x													
Flu-PRO Survey Instrument			x[11]	x	x	x	x	x	x	x	x					x[11]	x[11]
Collect Flu-PRO Survey Instrument												x					
Adverse event review[12]				x	x	x	x	x	x	x	x	x	x			x[22]	x[22]
SAE review				x	x	x	x	x	x	x	x	x	x	x	x	x	X
ECG (12 lead)	x[13]								x[13]								
CXR (PA and lateral)	x																
Blood for HIV, HBV, HCV	x																
Serum HCG	x																
Urine HCG[14]	x	x															
Urine toxicology[15]	x	x															
Blood for Safety Lab Evaluations (Screen)[16]	x																
Blood for Safety Lab Evaluations (Follow-up)[17]					x		x				x					x[22]	
Sera for cytokine and/or antibody assays[18]		x			x		x		x		x	x	x	x			
Blood for ASC (ELISpot; fresh PBMC)[19]							x		x		x						
Blood for innate and/or T and B immunophenotyping (cryopreserved PBMC)[19]		x					x		x		x	x	x	x			

Evaluation	**Enrollment/Screening Study Visit 00A Day -30 to -3**	**Inpatient Study Visit 00B Day -2**	**Inpatient Study Visit 00C Day -1**	**Inpatient Study Visit 1 Day 1**	**Inpatient Study Visit 2 Day 2**	**Inpatient Study Visit 3 Day 3**	**Inpatient Study Visit 4 Day 4**	**Inpatient Study Visit 5 Day 5**	**Inpatient Study Visit 6 Day 6**	**Inpatient Study Visit 7 Day 7**	**Inpatient Study Visit 8 Day 8**	**Follow-up Study Visit 9 Day 15±3**	**Follow-up Study Visit 10 Day 29±3**	**Follow-up Study Visit 11 Day 61±5**	**Final Study Visit 12 Day 91 ± 7**	**Early Termination Visit**	**Unscheduled Visit**
Blood for T and B immunology (cryopreserved PBMC)		x					x		x		x	x	x	x		x[22]	x[22]
Blood for transcriptomics (whole blood)		x		x[20]	x		x				x	x				x[22]	x[22]
NP swab for qualitative & quantitative respiratory		x	x		x	x	x	x	x	x	x						
NLF for cytokine and/or antibody assays		x			x		x		x		x	x	x	x		x[22]	x[22]
NLF for other immunology (immunophenotyping)		x			x		x		x		x					x[22]	x[22]
HLA Typing														x		x[22]	x[22]

1. Visit type: c=clinic, i=inpatient unit, p=phone call
2. Eligibility criteria may change from the day of enrollment to Day -3. To avoid admitting subjects with new exclusion criteria into the inpatient unit, the eligibility criteria will be reviewed and confirmed before the inpatient stay.
3. Subjects will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the inpatient unit before challenge virus is administered.
4. Eligible subjects will be admitted to the inpatient unit on Day -2 (which is two days prior to the planned challenge).
5. Subjects will remain in the inpatient unit for a minimum of seven days after the date of challenge. Subjects will leave the unit after they meet the following discharge criteria: two consecutive negative NP swabs (that are performed on Day 7 or thereafter) for influenza A by qualitative RT-PCR performed by the local clinical laboratory, are afebrile, have $SpO_2 \geq 95\%$ on room air, show no moderate or severe influenza signs or symptoms by clinical evaluation, and are clinically and hemodynamically stable for 48 hours. Subjects who do not meet discharge criteria on Day 8 will remain in the inpatient unit until the criteria are met. Study procedures for the inpatient unit will be the same as on Day 8, except for no additional blood draws unless clinically indicated.
6. A single dose of baloxavir marboxil will be offered to all subjects who do not have two consecutive negative NP swabs (on Day 7 or thereafter) for influenza A by qualitative RT-PCR.

7. Height and weight will be measured at initial Screening. Weight will be documented a second time on admission to the inpatient unit. BMI calculation for the purpose of Eligibility will be based on the first measured weight. BMI for the purpose of analyses will be calculated with the second measured weight.
8. Clinical evaluation (including lung, heart, oral/pharyngeal, and neck exams), and symptom evaluation daily while in the inpatient unit.
9. Oral temperature, blood pressure, pulse, and respiratory rate will be assessed three times per day while the subjects are awake, and as clinically indicated.
10. Peripheral mixed venous oxygen saturation (SpO_2) on room air at screening, on admission, and when oral temperature, blood pressure, pulse, and respiratory rate are assessed through the inpatient stay.
11. Training on Flu-PRO Survey Instrument and Validation Diary for evaluation of severity score. Self-assessment using Flu-PRO and diary to be performed in the morning pre-challenge on Day 1, and at approximately 5 PM through day 14 during the inpatient stay and following discharge to home.
12. Unsolicited AEs will be monitored for approximately 30 days post challenge. Review of Flu-PRO for indicators of possible severe influenza associated illness (signs, symptoms, or lab findings) not consistent with MMID.
13. ECG to be performed at screening, on Day 6, and as clinically indicated. VTEU sites will consult a cardiologist if ECG is abnormal to determine clinically necessary work up.
14. Among female subjects of childbearing potential, a repeat urine pregnancy test will be performed locally before CXR performed if > 7 days have passed since the negative serum pregnancy test was drawn.
15. Negative drug urine toxicology result (amphetamines, cocaine, and opiates,) required on screening performed at the Central Lab, and (amphetamines, cocaine, opiates, and cannabinoids) on admission to the challenge unit performed locally.
16. Screening Safety Labs: white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, alanine transaminase (ALT), and creatinine (Cr).
17. Follow-Up Safety Labs: Days 2, 4, and 8, and as clinically indicated: white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), and creatinine (Cr).
18. Including HAI, MN and NAI antibody titers at admission to the inpatient unit (Day -2) and Visits 8,10 and 11.
19. Will be drawn from at least a subset of participants for local testing
20. Blood draw to occur prior to challenge
21. Multiplex respiratory virus assay to be performed at each site. Positive results prior to influenza virus challenge will result in subject exclusion from the study. No respiratory virus testing will be done on the day of challenge.
22. If indicated.

Appendix B. Venipuncture Volumes (mL)

Table 13: Venipuncture Volumes (all volumes are approximate)

Evaluation	Enrollment/Screening Study Visit 00A Day -30 to -3	Inpatient Study Visit 00B Day -2	Inpatient Study Visit 00C Day -1	Inpatient Study Visit 1 Day 1	Inpatient Study Visit 2 Day 2	Inpatient Study Visit 3 Day 3	Inpatient Study Visit 4 Day 4	Inpatient Study Visit 5 Day 5	Inpatient Study Visit 6 Day 6	Inpatient Study Visit 7 Day 7	Inpatient Study Visit 8 Day 8	Follow-up Study Visit 9 Day 15±3	Follow-up Study Visit 10 Day 29±3	Follow-up Study Visit 11 Day 61±5	Final Study Visit 12 Day 91 ± 7	Total Volumes
Blood for Safety Lab Evaluations (Screen)	15[1]															15
Blood for Safety Lab Evaluations (Follow-up)					10[2]		10[2]				10[2]					30
Blood for HLA typing														0[3]		0
Sera for antibody and cytokine assays		20[4,5]			5[5]		5[5]		5[5]		20[4,5]	5[5]	20[4]	20[4]		100
Blood for ASC (ELISpot; fresh PBMC)[6]							15[7]		15[7]		15[7]					45
Blood for innate and/or T and B immunophenotyping (cryopreserved PBMC)[6]		8[8,9]					8[8,9]		8[8,9]		8[8,9]	8[8,9]	8[9]	8[9]		56
Blood for T and B immunology (cryopreserved PBMC)		48[10]					32[10]		32[10]		32[10]	40[10]	40[10]	40[10]		264
Blood for transcriptomics (whole blood)		2.5		2.5[11]	2.5		2.5				2.5	2.5				15
Total (fresh PBMC subset)	15	78.5	0	2.5	17.5	0	72.5	0	60	0	87.5	55.5	68	68	0	525
Total (subset without fresh PBMC)	15	78.5	0	2.5	17.5	0	57.5	0	45	0	72.5	55.5	68	68	0	480

1. Safety Lab Evaluations (Screen): Hepatitis B surface antigen (HBsAg), hepatitis C antibodies (anti-HCV), human immunodeficiency virus (HIV) testing, and serum HCG (for women subjects of childbearing potential), white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), and creatinine (Cr).
2. Blood for Safety Lab Evaluations (Follow-up): white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, and alanine transaminase (ALT), and creatinine (Cr).
3. HLA testing may be performed on blood already drawn up to this timepoint with no increase in blood volume drawn
4. Sera tested for antibodies
5. Sera tested for cytokines
6. Blood draw and testing will be done in at least a subset of participants
7. A/Bethesda/MM2/H1N1-specific plasmablast responses (ASC) H1 and N1
8. Immunophenotyping of innate cells
9. Immunophenotyping of T and B cell subsets (including cTfh)
10. A/Bethesda/MM2/H1N1-specific responses in T and B cell subsets (including cTfh)
11. Drawn prechallenge

Appendix C. High-Risk Medical Conditions for Severe Influenza

People with any of the following medical conditions are at increased risk for serious complications from influenza virus infection [56].

- Asthma
- Neurologic and neurodevelopment conditions
- Blood disorders (such as sickle cell disease)
- Chronic lung disease (such as chronic obstructive pulmonary disease [COPD] and cystic fibrosis)
- Endocrine disorders (such as diabetes mellitus)
- Heart disease (such as congenital heart disease, congestive heart failure and coronary artery disease)
- Kidney disorders
- Liver disorders
- Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders)
- Obesity with a body mass index [BMI] of 40 or higher
- Weakened immune system due to disease (such as people with HIV or AIDS, or some cancers such as leukemia) or medications (such as those receiving chemotherapy or radiation treatment for cancer, or persons with chronic conditions requiring chronic corticosteroids or other drugs that suppress the immune system)
- Pregnant women

Appendix D. Flu-PRO Survey Instrument and Validation Diary

Participant ID: _____ Participant Initials: _____ Date: ____/_____/_____

FLU-PRO©

People experience the flu in different ways. We would like to know about the symptoms you have been experiencing during the past 24 hours. For each symptom, please mark one box ☐ under the response that best matches your experience. Mark the "Not at all" box, if you did not have that symptom in the past 24 hours.

What time is it? _____ AM / PM (please circle)

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Runny or dripping nose	☐	☐	☐	☐	☐
Congested or stuffy nose	☐	☐	☐	☐	☐
Sinus pressure	☐	☐	☐	☐	☐
Scratchy or itchy throat	☐	☐	☐	☐	☐
Sore or painful throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Teary or watery eyes	☐	☐	☐	☐	☐
Sore or painful eyes	☐	☐	☐	☐	☐
Eyes sensitive to light	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Felt nauseous (feeling like you wanted to throw-up)	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐

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Participant ID: _____ Participant Initials: _____ Date: ____/_____/_____

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Lack of appetite	☐	☐	☐	☐	☐
Sleeping more than usual	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Felt cold	☐	☐	☐	☐	☐
Felt hot	☐	☐	☐	☐	☐
Sweating	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	Never	Rarely	Sometimes	Often	Always
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

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ADDITIONAL DAILY DIARY ITEMS FOR FLU-PRO VALIDATION STUDY

Items to be asked in the daily diary through to Day 14 along with the FLU-PRO items.

1. Did you take any medication for your flu symptoms today? (Please select one response only)
   - ☐1 Yes
   - ☐0 No

2. Do you have asthma, COPD (chronic obstructive pulmonary disease) or both?
   - ☐1 Yes
   - ☐0 No

3. [Only asked if answer to the question above is "yes"]. Did you use any rescue medication today for your asthma or COPD? (Please select one response only)
   - ☐1 Yes
   - ☐0 No

4. Overall, how severe were your flu symptoms today? (Please select one response only)
   - ☐0 No flu symptoms today
   - ☐1 Mild
   - ☐2 Moderate
   - ☐3 Severe
   - ☐4 Very severe

5. Overall, how were your flu symptoms today compared to yesterday? (Please select one response only)
   - ☐1 Much better
   - ☐2 Somewhat better
   - ☐3 A little better
   - ☐4 About the same
   - ☐5 A little worse
   - ☐6 Somewhat worse
   - ☐7 Much worse

6. How much did your flu symptoms interfere with your usual activities today? (Please select one response only)
   - ☐1 Not at all
   - ☐2 A little bit
   - ☐3 Somewhat
   - ☐4 Quite a bit
   - ☐5 Very much

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7. Have you returned to your usual activities today?
   - ☐1 Yes
   - ☐0 No

8. In general, how would you rate your physical health today? (Please select one response only)
   - ☐5 Excellent
   - ☐4 Very Good
   - ☐3 Good
   - ☐2 Fair
   - ☐1 Poor

9. Have you returned to your usual health today?
   - ☐1 Yes
   - ☐0 No