A Multicenter, Blinded, Randomized, Placebo-Controlled, Dose-Ranging Influenza Challenge Study in Healthy Adult Volunteers to Determine the Optimal Infection Dose and Safety of a Recombinant H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) Influenza Challenge Virus

Short Title: A/Texas/71/2017 (H3N2) Dose-ranging Challenge Study

DMID Protocol Number: DMID 20-0005

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National Institutes of Allergy and Infectious Diseases,
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STATEMENT OF COMPLIANCE

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research. The IRB/IEC must be registered with OHRP as applicable to the research.

The study will be carried out in accordance with the following as applicable:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations: 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), and/or 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice, and the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and DMID
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

The signature below provides the necessary assurance that this study will be conducted according to all stipulations of the protocol including statements regarding confidentiality, and according to local legal and regulatory requirements, US federal regulations, and ICH E6(R2) Good Clinical Practice (GCP) guidelines.

Site Investigator Signature:

Signed: _____ Date: _____

Name
Title

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1.1 Synopsis

Rationale for Proposed Clinical Study

Influenza is a seasonal respiratory disease that is a leading cause of hospitalizations, missed work, missed school and economic disruption. While there are currently a number of seasonal influenza vaccines available across numerous vaccine platforms, efforts are still underway to develop either an improved seasonal vaccine with greater efficacy, or a universal vaccine. The human challenge model has proven essential to determine the effectiveness of a vaccine in a controlled setting. This study is designed to determine the optimal infectious dose of the H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus challenge strain for use in future Phase I clinical trials evaluating vaccine candidates.

Study Design

The study will enroll and challenge up to 106 (plus approximately 8 shams) healthy adult volunteers with the H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus challenge strain. Subjects will be pre-screened for study inclusion to have serological HAI antibody titers of ≤1:40 against the clinical challenge strain. Eligible participants will be enrolled sequentially into dosing cohorts and will be randomly assigned to receive a single dose of either placebo (sham inoculum) or a virus dose between 10^4 to 10^6 $TCID_{50}$ (in an allocation of 1:12 to 1:17). Dose titration will be conducted under an adaptive escalation schedule (Figure 1) whereby dosing will start at the lowest dose (10^4 $TCID_{50}$) and only escalate to the next dose if a pre-determined infection and symptomatic attack rate are not met and the dose is determined to be safe and no pre-defined halting rule is met. The attack rate (AR), defined as the percentage of subjects meeting shedding and symptom criteria for symptomatic influenza virus infection (see clinical case definition below), will be determined for each challenge dose group in order to identify the optimal infectious dose (55%-80% AR). This adaptive, dose-ranging approach allows adjustments to challenge dose group size and escalation dose schedule to be informed by the AR and safety results.

Study Objectives

Primary Objectives:

1. To determine the optimal infectious dose[1] of a recombinant influenza virus (A/Texas/71/2017 (H3N2), clade 3C3a) to be used as a clinical challenge strain in future vaccine efficacy or intervention studies as assessed by viral shedding and clinical symptoms

2. To describe viral detection by quantitative and qualitative RT-PCR from study subjects at baseline and post-challenge

3. To document clinical symptoms from self-reported surveys and standardized symptom scales at baseline and post-challenge

Secondary Objectives:

1. To assess the safety profile of a live recombinant influenza strain (A/Texas/71/2017 (H3N2), clade 3C3a) following challenge in healthy adult volunteers

2. To describe the host serum hemagglutination inhibition and microneutralization antibody responses at baseline and post-challenge

3. To describe anti-HA-stalk antibody titer at baseline and post-challenge

Exploratory Objectives:

1. To describe the host serum neuraminidase inhibiting antibody responses at baseline and post-challenge

2. To describe the host immune responses (innate, serum antibody, nasal IgA) at baseline and post-challenge in at least a subset of subjects.

3. To describe physiological responses from an influenza infection as assessed by wearable devices at baseline and post-challenge

4. To describe systemic transcriptional responses at baseline and post-challenge in at least a subset of subjects

5. To compare the clinical features of symptomatic RT-PCR-positive and RT-PCR-negative illness post-challenge

6. To explore alternative case definitions (e.g. MMID) for symptomatic RT-PCR-positive influenza virus infection

7. To determine the symptomatic background within uninfected participants and between sites

8. To evaluate the plasmablast response post-challenge in a subset of study subjects

9. To describe mucosal and systemic human immune cells in blood and NLF and/or nasal curettage at baseline and post-challenge in at least a subset of study subjects

10. To describe T cell activation in cells from nasal curettage in a subset of study subjects

11. To assess the association of human leukocyte antigen (HLA) class I and II alleles with clinical, immune and viral responses in at least a subset of subjects

12. To evaluate cellular immune response at baseline and post-challenge in at least a subset of subjects

13. To describe viral detection by quantitative culture from study subjects at baseline and post-challenge

[1]*The optimal infectious dose is the minimal infectious dose that is considered safe and maximizes the AR and symptoms among the doses assessed*

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 7 days after challenge AND until they have no viral shedding[1], determined by qualitative RT-PCR beginning on Study Day 6

   [1]*No viral shedding is defined as two negative RT-PCR tests 12 or more hours apart*
4. Healthy[2] males and non-pregnant, non-breastfeeding females aged ≥ 18 and < 46 years of age at enrollment

   [2]*Healthy is defined in inclusion criteria #11.*

   NOTE: Female subjects of childbearing potential must have a negative serum pregnancy test at screening, a negative urine pregnancy test upon admission to the confinement unit AND a negative pregnancy test before any CXR (if ≥ 7 days have passed since a serum pregnancy test).
5. Women of childbearing potential[3] must agree to use or have practiced true abstinence[4] or use at least one acceptable primary form of contraception[5] for at least 30 days prior to challenge

   [3]*Not of childbearing 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).*

   [4]*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).*

   [5]*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. Must use at least one acceptable primary form of contraception for at least 30 days prior to challenge and at least one acceptable primary form of contraception during the remainder of the study or approximately 57 days after confinement.*

   NOTE: These criteria are applicable to female subjects in a heterosexual relationship

AND of child-bearing potential. These criteria do not apply to subjects in a same sex relationship.

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

   [6]Non-habitual smokers are those who smoke no more than four cigarettes, other tobacco products, e-cigarettes (to include vaping and Juuling products) or marijuana in a week 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 within the last 2 years and agrees to abstain from alcohol for at least one week before admission and throughout the confinement period

8. No self-reported or known history of restricted drug use for at least 30 days prior to challenge and agrees to abstain from restricted drugs throughout the confinement period

9. Negative drug urine toxicology result on screening (i.e., amphetamines, cocaine, and opiates) and on admission to the confinement unit (i.e., amphetamines, cocaine, and opiates)[8]
   [8]Select drug use may be allowed at Investigator's discretion (e.g., prescribed amphetamines for ADHD)

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

    [9]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)

11. In good health[10], and do not have clinically significant medical, psychiatric, chronic or intermittent health conditions including those listed in Exclusion Criteria (Section 5.2)

    [10]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 5.2).

    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 5.2) 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.

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 ≤ 18
    - Oral temperature is less than 100.6°F
13. Eligibility laboratory values (WBC, Absolute Lymphocyte Count, Hgb, PLTs, ALT and Cr) are within acceptable parameters[11]

    [11]*Labs within normal range or grade 1 abnormalities deemed not clinically significant by a study investigator are considered acceptable*
14. Body mass index (BMI) >18.5 and <40 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[12]

    [12]*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
17. Negative respiratory virus panel by BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or by Luminex xTAG® on Day -2, and Day -1
18. Negative RT-PCR test for SARS-CoV 2 on screening and Day -2
19. HAI antibody titer ≤1:40 against influenza A/Texas/71/2017 (H3N2) at screening
20. Receipt of the recommended number of doses of an EUA authorized or licensed COVID-19 vaccine product ≥ two weeks prior to confinement

Exclusion Criteria

1. Presence of self-reported or medically documented significant medical or psychiatric condition(s)[1]

   [1]*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 [Inhaled, oral or intravenous (IV) corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics] or any treatment for respiratory disease exacerbations (e.g., asthma exacerbation) within the last 5 years*
   b. *Presence of any febrile illness or symptoms suggestive of a respiratory infection within two weeks prior to challenge*
   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)*
f. *Presence of an autoimmune disease.*
g. *Immunodeficiency of any cause.*
h. *History of diabetes.*

2. Presence of immunosuppression or any medications that may be associated with impaired immune responsiveness[2]

   [2]*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 6.5).*

3. Known allergy or intolerance to treatments for influenza (including any neuraminidase inhibitors or baloxavir marboxil).
4. Known allergy to two or more classes of antibiotics (*e.g.*, penicillins, cephalosporins, fluoroquinolones, or glycopeptides).
5. Known allergy to excipients[3] in the challenge virus inoculum.

   [3] *Sucrose, KH_2PO_4, K_2HPO_4, L-glutamic acid (in SPG diluent)*

6. Receipt or planned receipt of any investigational drug/investigational vaccine/licensed vaccine, except for a licensed or emergency use authorized COVID-19 vaccine product, within 30 days prior to the date of challenge.
7. Prior enrollment in an influenza virus challenge study with an influenza virus of the same subtype within the past 2 years.
8. Currently enrolled in any investigational study or intends to enroll in such a study within the ensuing study period.[4]

   [4]*Co-enrollment in an observational study, an investigational study in a follow-up/post-vaccination stage, or a study involving a licensed drug or biologic may be allowed at the investigator's discretion.*

9. Receipt of any influenza vaccine four months prior to challenge or plans to receive influenza vaccine during the study (approximately 57 days after challenge).
10. History of a previous severe allergic reaction to any drug or biologic with generalized urticaria, angioedema, or anaphylaxis.
11. Receipt of blood or blood products during the six months prior to the planned date of challenge.

12. Plans to donate blood or blood products during the study (approximately 102 days).
13. Any condition (including medical and psychiatric conditions) that, in the opinion of the Investigator, might interfere with the safety of the subject and/or study objectives.
14. An ongoing symptomatic condition for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan e.g., ongoing fatigue without a diagnosis.
15. Known close contact with anyone known to have or suspected to have a respiratory viral illness within 7 days prior to challenge.
16. Significant abnormality altering anatomy of nose/nasopharynx (including significant nasal polyps), clinically significant nasal deviation, or nasal/sinus surgery within 180 days prior to challenge.
17. History in the last five years of chronic or frequent intermittent sinusitis.
18. Recent history (180 days) of epistaxis or anatomic or neurologic abnormality impairing the gag reflex or contributing to aspiration.
19. Currently using an internal cardiac device such as a pacemaker or other implanted electronic medical devices.

Study Phase

- Phase 1

Study Population

Healthy males and non-pregnant, non-breastfeeding females aged ≥ 18 and < 46 years of age with a serum HAI antibody titer of ≤ 1:40 against influenza A/Texas/71/2017 (H3N2), clade 3C3a.

Study Sites

There will be two US sites.

Study Intervention

The study is designed to assess the influenza AR following challenge with recombinant H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus at sequentially increasing doses from 10^4, 10^5, and up to 10^6 $TCID_{50}$. Initially, the trial will start with a challenge cohort receiving a single dose of 10^4 $TCID_{50}$ of recombinant H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus administered intranasally. Subsequent cohorts will receive either the same dose or the dose will escalate to the next highest dose in a stepwise manner depending upon the AR observed. Within each challenge cohort, subjects will be randomized to receive either challenge virus or an intranasally administered sham placebo. For challenge cohorts 1A, 1B, 1C, 2A, 2B, and 2C the study will randomize subjects 12:1 targeted to challenge virus or sham placebo. For challenge cohorts 3A and 3B the study will randomize subjects 17:1 targeted to challenge virus or sham placebo.

Study Duration

This study will last for 7.5 months - one year depending upon the number of challenge cohorts enrolled given the adaptive dose escalation design.

Participant Duration

An individual subject will complete the study in about 102 days, from screening at Day -45 to -3 to follow-up on Day 57 ± 7.

Safety

Between each challenge cohort, an Internal Safety Review Committee (ISRC) will review blinded subject data from Days 1 through Day 8 to assess if any of the halting criteria (Section 7.1.1), including the occurrence of serious and severe influenza illness (Section 2.3.1), is met. If none of the halting criteria is met, as assessed by the ISRC, the study can proceed to enroll the next challenge cohort according to the adaptive trial design. If any of the halting criteria are met, an SMC will review the subject safety data in an ad hoc meeting.

1.2 Schedule of Activities (SoA)

Table 1. Schedule of Activities (SoA)

	Study Screening	Pre-Challenge		Viral Challenge	Post-Challenge												
		Confinement period											Follow-up[29]				
Study Days	D -45 to -3	D -2	D -1	D1	D 2	D 3	D 4	D 5	D6	D 7	D8	D9+	D15 ± 3	D29 ± 5	D57 ± 7	E[1]	U[2]
Visit Number	00A	00B	00C	1	2	3	4	5	6	7	8		9	10	11		
Type of Visit[3]	C	I	I	I	I	I	I	I	I	I	I	I	C	C	C	C	C
Clinical Procedures																	
Informed consent	X																
Review/confirm eligibility criteria[4]	X	X	X	X													
Review/confirm informed consent[5]		X		X													
Confinement begins		X[6]															
Confinement period		X	X	X	X	X	X	X	X	X	X	X					
Discharge from confinement unit											X[7]	X					
Initiate treatment course with antiviral											X[8]					X[8]	
Demographics	X																
Medical History	X	X															
Height[9]	X																
Weight[9]	X																
Concomitant medications[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam	X																
Targeted physical exam[11]		X	X	X	X	X	X	X	X	X	X	X[24]	X	X	X	X[24]	X[24]
Vital signs[12]	X	X	X	X	X	X	X	X	X	X	X	X[24]	X	X	X	X[24]	X[24]
SpO_2[13]	X	X	X	X	X	X	X	X	X	X	X	X[24]	X	X	X		
Influenza challenge				X													
FLU-PRO survey instrument PM[14]		X	X	X	X	X	X	X	X	X	X	X	X				
Modified Jackson Score AM and PM[15]		X	X	X	X	X	X	X	X	X	X	X	X				
Adverse event review[16]				X	X	X	X	X	X	X	X	X[24]	X	X		X[24]	X[24]

	Study Screening	Pre-Challenge		Viral Challenge	Post-Challenge												
		Confinement period											Follow-up[29]				
Study Days	D -45 to -3	D -2	D -1	D1	D 2	D 3	D 4	D 5	D6	D 7	D8	D9+	D15 ± 3	D29 ± 5	D57 ± 7	E[1]	U[2]
Visit Number	00A	00B	00C	1	2	3	4	5	6	7	8		9	10	11		
Type of Visit[3]	C	I	I	I	I	I	I	I	I	I	I	I	C	C	C	C	C
SAE review				X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG (12 lead)	X[17]								X[17]								
Chest X-Ray[18]	X																
Blood for HIV, HBV, and HCV	X																
Serum HCG	X																
Urine HCG[18]		X															
Urine toxicology[19]	X	X															
Safety screening labs[20]	X																
HAI Serology screen	X																
Safety follow-up labs[21]					X		X				X	X[24]				X[25]	X[24]
Research Laboratory Procedures																	
Blood - for plasma and PBMC for T- and B- cell immunology			X								X[24]		X	X	X	X[26]	X[26]
Blood - Serum for antibody and cytokine assays[22]		X	X		X	X	X	X	X	X	X		X	X	X	X[26]	
Plasmablasts											X						
Blood – PBMC for immunophenotyping[31]			X		X		X		X		X			X			
Blood - RNA transcriptomics		X	X		X	X	X	X	X	X	X	X[24]	X			X[26]	X[26]
Blood – DNA – collected for genetic testing			X														
Nasopharyngeal swab(s) for viral culture and/or qualitative[23] and quantitative RT-PCR	X	X	X		X	X	X	X	X	X	X	X					
Nasal absorptive matrices and nasal lavage for antibody and other assays		X	X		X	X	X	X	X	X	X		X	X	X	X[26]	X[26]

	Study Screening	Pre-Challenge		Viral Challenge	Post-Challenge												
		Confinement period											Follow-up[29]				
Study Days	D -45 to -3	D -2	D -1	D1	D 2	D 3	D 4	D 5	D6	D 7	D8	D9+	D15 ± 3	D29 ± 5	D57 ± 7	E[1]	U[2]
Visit Number	00A	00B	00C	1	2	3	4	5	6	7	8		9	10	11		
Type of Visit[3]	C	I	I	I	I	I	I	I	I	I	I	I	C	C	C	C	C
Curettage – nasal mucosa for t cell activation[30]		X											X	X	X	X[26]	X[26]
Wearable Devices																	
Garmin and/or Oura Ring[27]	X[28]	X	X	X	X	X	X	X	X	X	X	X					
Faros device[27]		X	X	X	X	X	X	X	X	X	X	X					

1. E=Early termination visit
2. U=Unscheduled visit
3. Visit type: C = Clinic, I = Inpatient
4. Eligibility criteria may change from the day of screening to Study Day -3. To avoid admitting subjects to confinement with new exclusion criteria, the eligibility criteria will be reviewed and confirmed before the confinement stay.
5. Subjects will have approximately a 48-hour window to decide if they would like to drop out of the study and leave the confinement unit before challenge virus is administered.
6. Eligible subjects will be confined under respiratory isolation beginning on Study Day -2 (which is two days prior to the planned challenge).
7. Subjects will remain in the confinement 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 collected 12 hours apart (that are performed on Study Day 6 or thereafter) for influenza A by qualitative RT-PCR performed by the local clinical laboratory, are afebrile (< 100.6°F/38.1°C), 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 Study Day 8 will remain in the confinement unit until the criteria are met. Study procedures for the confinement unit will be the same as on Study Day 8, except for no NLF collection or additional blood draws unless clinically indicated.
8. A treatment course of oseltamivir phosphate or baloxavir marboxil will be provided to all subjects who do not have two consecutive negative NP swabs by qualitative RT-PCR for influenza by Study Day 6. Treatment will begin as early as Study Day 6, or thereafter if indicated, or earlier if terminates study prior to Study Day 8.

9. Height and weight will be measured at Screening.
10. Concomitant medications taken within 90 days prior to signing the ICF are collected.
11. Clinical evaluation (including oral/pharyngeal, neck, lung and heart exam), and symptom evaluation daily while in the confinement unit. An otoscopic exam will be performed at baseline and as needed.
12. Oral temperature, blood pressure, pulse, and respiratory rate will be assessed approximately every 8 hours while in the confinement unit (from 12:00AM – 11:59PM), while the subjects are awake, and as clinically indicated.
13. Peripheral 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 confinement period and at follow-up visits.
14. Self-assessment using FLU-PRO and Validation Diary to be performed after 3:00 pm on the Day -2 and after 3:00 pm each day of confinement and through Day 15.
15. Modified Jackson Score to be obtained on the evening of Day -2 and in the morning and afternoon of each day of confinement (Approximately at 8:00 AM and after 3:00 PM) and on Day 15 during visit. Assure AM assessment on Study Day 1 is performed pre-challenge.
16. Unsolicited AEs will be monitored for approximately 28 days post challenge. Review of FLU-PRO for indicators of possible severe influenza associated illness (signs, symptoms, or lab findings).
17. ECG to be performed at screening, on Study Day 6, and as clinically indicated. Sites will consult a cardiologist if ECG is abnormal to determine clinically necessary work up.
18. 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.
19. Negative drug urine toxicology result (amphetamines, cocaine, and opiates) required on screening and (amphetamines, cocaine, opiates) on admission to the challenge unit, unless the drug is deemed acceptable by the Investigator.
20. Screening Safety/Eligibility Labs: white blood cells (WBCs), absolute lymphocyte count, hemoglobin, platelets, alanine transaminase (ALT), and creatinine (Cr).
21. Follow-Up Safety Labs: Study 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).
22. Including HAI, MN and NAI antibody titers on Visits 00C (Day -1) 8, 9, 10 and 11.
23. Multiplex respiratory virus assay to include SARS-CoV-2 to be performed at each site at the time points listed above. 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. Quantitative PCR will not be done at screening.
24. If indicated.
25. If terminates study < Study Day 8 and procedure not performed on day study terminated.

26. If terminates study early and procedure not scheduled to be performed on day study terminated or if missed study procedure on scheduled day.
27. To achieve baseline data for physiological biomarkers (e.g., heartrate variability, sleep patterns, etc.).
28. If the Garmin and/or Oura ring is not able to be distributed at screening this will not be a protocol deviation.
29. Follow-up visits can be conducted by phone if in-person visit is not feasible.
30. Nasal curettage will be an optional procedure.
31. At UMB, on Study Days -1, 2, 4, 6 and 8, $1x10^6$ freshly isolated PBMCs will be used to stain by mass cytometry. These specimens will be stained in conjunction with NLF cells isolated from individuals whose NLF cell counts trigger mass cytometry staining (>50,000 cells). No additional blood volumes or additional blood draws will be required.

1.3 Study Schema

Figure 1. Adaptive Dosing Schedule

See Section 4.1 for a detailed explanation of the study schema.

2. INTRODUCTION

2.1 Study Rationale

Influenza is a seasonal respiratory disease that is a leading cause of hospitalizations, missed work, missed school and economic disruption. While there are currently a number of seasonal vaccines available across numerous vaccine platforms, efforts are still underway to develop either an improved seasonal vaccine with greater efficacy, or a universal vaccine. The human challenge model has proven a valuable tool to determine the effectiveness of a vaccine in a controlled setting. This study is designed to determine the optimal infectious dose of the H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus challenge strain for use in future phase 1 clinical trials evaluating vaccine candidates.

2.2 Background

2.2.1 Purpose of Study

Despite current vaccination strategies and availability of antivirals, influenza remains an important cause of worldwide morbidity and mortality. Those particularly at risk include older adults, young children, the immunocompromised, and individuals with chronic respiratory, cardiac, or metabolic diseases. The young adult population was particularly vulnerable during the 2009 H1N1 pandemic. Therefore, improved prevention and management approaches for seasonal and pandemic influenza across all populations are urgently needed, including the development of new universal vaccines that offer broad and robust protection.

One of the key components 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] After recent completion of the DMID sponsored 18-0010 in-patient study (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) BB-IND 19213, the Collaborative Influenza Vaccine Innovation Centers (CIVICs) are now planning dose-ranging studies for recombinant H3N2 and other H1N1 strains to be provided by DMID. The CIVICs program is part of NIAID's universal influenza vaccine strategic plan and aims to support development of novel influenza vaccines that provide broader and more robust protection and immunity through coordinated multi-disciplinary efforts toward discovery, product development, and pre-clinical and clinical evaluation in human challenge studies.[2]

Human challenge models provide a powerful tool to assess prevention and treatment options for influenza in a shorter timeframe and with a smaller number of subjects compared to typical clinical trials, using a well-characterized virus strain. The controlled time and inoculum of virus exposure in challenge studies allow for targeted collection of clinical samples, including prior to infection and in short timeframes 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 evaluated based on current regulatory criteria for licensure of inactivated influenza vaccines. In addition, human challenge models offer the unique opportunity to further study influenza pathogenesis, host immune responses, and correlates of protection, which remain

unclear. Such experimental models are conducted in a well-controlled challenge setting in which virus exposure, infection rate, virus shedding, and controlled sampling are monitored in real-time. Such parameters are next to impossible to control for in a "real world" setting. Importantly, the setting of a controlled experimental model offers the opportunity to simultaneously evaluate the clinical, molecular, physiological and immunological outcomes and responses across the time course of disease and treatment.

The overall objective of the present study is to conduct a dose-ranging human challenge study with two CIVICs clinical programs to determine the safety and optimal infectious dose of a recombinant H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza virus. The study will enroll and challenge up to 106 persons with live virus (plus approximately 8 persons with shams) healthy adult volunteers who will be prescreened for study inclusion to have serological HAI antibody titers of ≤1:40 against the challenge strain. Eligible participants will be enrolled sequentially into challenge cohorts and will be randomly assigned to receive a single dose of either sham inoculum or a virus dose between 10^4 to 10^6 $TCID_{50}$. Dose titration will be conducted under an adaptive escalation schedule (**Figure 1**) whereby dosing will start at the lowest dose (10^4 $TCID_{50}$) and only escalate to the next dose if a pre-determined symptomatic influenza attack rate is not met and the dose is determined to be safe and no pre-defined halting rule is met. In addition to safety, the attack rate (AR), defined as the percentage of subjects meeting shedding and symptom criteria to be classified as having symptomatic influenza infection (see clinical case definition in Section 4.1), will be determined for each challenge dose group in order to identify the optimal infectious dose (55%-80% AR). This adaptive dose ranging approach allows adjustments to challenge dose group size and escalation dose schedule to be informed by the AR and safety results.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

The potential risks of participating in this trial are listed below.

Symptoms and Complications of Influenza Illness

Minor symptoms of common 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
- Feeling dizzy
- Fever
- Head Congestion

- Headache
- Lack of appetite
- Swollen lymph nodes
- 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
- Stomachache
- Sweating
- Teary or watery eyes

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

Moderate complications

- Sinus infections
- Ear infections

Severe complications (for additional clarification on any of the below, please refer to study-specific SOP CIVICs 001)

- Pneumonia
- Severe dehydration
- Severe bronchitis
- Myocarditis
- Myocardial infarction
- Pericarditis
- Guillain Barré Syndrome/ Bell's Palsy
- Transverse myelitis
- Encephalitis
- Worsening of chronic health conditions
- Admission to hospital
- Hypoxemia/respiratory failure
- Arrhythmia/cardiac arrest
- Death

Though all subjects will be vaccinated, there is a risk of being exposed to SARS-CoV-2 as subjects will be evaluated at a screening visit and will be confined in an enclosed setting with other volunteers for an extended period of time. False negative RT-PCR upon study entry or introduction of COVID-19 by staff or other study personnel is possible.

Symptoms and Complications of COVID-19 Illness

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose
- Nausea or vomiting
- Diarrhea
- Respiratory failure
- Blood clots
- Death

There may be other risks, discomforts, or side effects that are unknown at this time. There is a small amount of risk to subjects that report they are in good health, but who have an unknown health problem at the time of screening. This trial will screen by medical history, ECG, Chest X-ray, vital signs and physical examination. Subjects will be screened for the presence of infection with HIV, hepatitis B and hepatitis C, and SARS-CoV-2. Women of childbearing potential will be screened to ensure they are not pregnant. Clinical screening labs for white blood cells (WBC), hemoglobin (Hgb), platelets (PLT), alanine aminotransferase (ALT), absolute lymphocyte count (ALC), and creatinine (Cr) will be drawn and obtained prior to enrollment and throughout the study. In addition, subjects will be pre-screened for serum HAI titer.

Risks of Nasal Lavage and Nasopharyngeal Swabs
Obtaining nasal lavage fluid or a nasopharyngeal swab can cause discomfort in the nares, nasopharynx, a gag reflex, epistaxis, watery eyes, or coughing at the time of specimen 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 aseptic 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 half-liter of blood (i.e., ~534 mL). The blood volumes anticipated for specimen collection are listed in Appendix C.

Risks of oseltamivir phosphate (TAMIFLU®) antiviral
Adverse events reported in at least 1% of adult and adolescent subjects treated with oseltamivir phosphate included nausea (10%), vomiting (9%), diarrhea (7%), bronchitis (2%), abdominal

pain (2%), dizziness (2%), headache (2%), cough (1%), insomnia (1%), vertigo (1%), and fatigue (1%).[3]

Influenza A virus isolates with reduced susceptibility to oseltamivir carboxylate have been recovered by serial passage of virus in cell culture in the presence of increasing concentrations of oseltamivir carboxylate, from clinical isolates collected during treatment with oseltamivir, and from viral isolates sampled during community surveillance studies. Reduced susceptibility of influenza virus to inhibition by oseltamivir carboxylate may be conferred by amino acid substitutions in the viral neuraminidase and/or hemagglutinin proteins. Changes in the viral neuraminidase that have been associated with reduced susceptibility to oseltamivir carboxylate.[3]

Risks of baloxavir marboxil (XOFLUZA™) 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%).[4]

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).[4]

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 2021-2022 influenza season vaccination through approximately 57 days post-challenge. If the influenza season begins before this time, the subject is at risk of developing influenza illness.

Risks Associated with the Garmin and Oura Wearable Devices
There are minor risks associated with the Garmin and Oura wearable device in this study. For the **Garmin Vivosmart 4 and Oura,** subjects with pacemakers or other internal electronic devices should consult a physician before using any heart rate monitor. This study excludes subjects with internal cardiac devices, minimizing this risk. The Garmin and Oura ring also emit green light and flash occasionally. Subjects with epilepsy or sensitivity to flashing lights should also consult their physician before use. The device operating temperature is -4 to 122°F while charging temperatures are 32 to 113°F. These temperature ranges should be observed in order to minimize heat and/or fire associated battery risks. Lithium-ion batteries should not be disassembled, removed or modified. They should not be exposed to fire or explosions, or there is a risk of fire, chemical burn, electrolyte leak, and/or injury.

Risks Associated with the Faros Wearable Device

The Bittium Faros 180 is an FDA approved Class II medical device designated for research use in adults. As long as the exclusion criteria are met (excluding those subjects with pacemakers or other implanted electronic medical devices), there are no known physical risks associated with wearing the Faros 180. Long-term ECG monitoring is an established procedure that carries minimal risks. Holter monitors, which are ambulatory ECG devices, are often worn for days or weeks to monitor heart rhythm. The only known physical risk related to wearing these types of sensors relates to skin irritation caused by the electrodes. The electrodes have a clear tape that allows for visual monitoring of skin condition. The participant will be instructed to temporarily remove the device if any redness or irritation develops because of sensitivity to the adhesive.

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 -45 to -3) and if deemed medically necessary as follow-up of an Adverse Event. Female subjects will undergo 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 -45 to -3), a repeat ECG at study Day 6, and additional ECGs if deemed medically necessary.

Risks of Genetic Testing
Genetic data may be stored and shared with other researchers through a controlled-access online repository, such as dbGaP. There may be a risk that genetic information while not linked with personal identifiable information, may potentially be reidentified and linked to a subject and could be misused for discriminatory purposes. However, such risk is perceived as low and state and federal laws provide some protections against genetic discrimination. Researchers who will have access to genetic information 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.

Risk of Loss of Confidentiality
There is also a risk for loss of confidentiality. In order to minimize this risk, data will be stored in a password protected computer database. Samples will be labeled with barcodes prior to storing with a unique identifier. All biological samples will be stored in secure and monitored freezers accessible only to key personnel with the appropriate access. Personnel working on this study will have undergone training for responsible conduct of research with human subjects and are listed on the delegation log/key personnel list and their relevant institutions.

2.3.2 Known Potential Benefits

There is no direct benefit to the subjects. There is potential benefit to the greater public health and future studies that may result from information gained from this study.

2.3.3 Assessment of Potential Risks and Benefits

The most cost effective and efficient method for assessing new influenza vaccines is through the use of controlled human influenza infection studies. Towards that end, it is necessary to determine the optimal dose of the influenza challenge strain as is being done in this study. The study will significantly add to knowledge about the best dose of the challenge virus to use in future studies evaluating new vaccine products. Although it is anticipated that subjects will develop mild influenza illness, the risk of any subject developing severe disease will be mitigated by carefully selecting subjects who are healthy. Subjects will be closely monitored for signs or symptoms of severe influenza illness and will be offered antiviral therapy for influenza and other medical care if such should occur. Likewise, subjects will be monitored carefully for any complications potentially arising from influenza. The potential risks therefore are thought to be acceptable given the potential benefits for society.

3. OBJECTIVES AND ENDPOINTS

The overall objective of the study is to determine the safety and optimal infectious dose of recombinant H3N2 (A/Texas/71/2017 (H3N2), clade 3C3a) influenza challenge virus.

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
Primary	
1. To determine the optimal infectious dose[1] of a recombinant influenza virus (A/Texas/71/2017 (H3N2), clade 3C3a) to be used as a clinical challenge strain in future vaccine efficacy or intervention studies as assessed by viral shedding and clinical symptoms	a. Percentage of subjects within a challenge dose group with detectable shedding in nasopharyngeal (NP) swab(s) by RT-PCR[2] over any 2 days beginning 24 hours post challenge through Day 8 and with symptom scores that meet the clinical case definition for symptomatic infection (Modified Jackson score). *See section 4.1 for the clinical case definition for symptomatic infection.*
2. To describe viral detection by quantitative and qualitative RT-PCR from study subjects at baseline and post-challenge	a. Percentage of subjects within a challenge dose group with detected viral shedding in NP swab(s) each day post-challenge from Day 2 through Day 8 using qualitative RT-PCR and quantitative RT-PCR b. Magnitude of virus shedding post-challenge in each subject defined as the peak viral load in NP swab(s) from day 2 through Day 8 post-challenge by quantitative RT-PCR c. Duration of viral shedding defined as the number of days from first positive RT-PCR to last positive RT-PCR where virus is detected in NP swab(s) by quantitative or qualitative RT-PCR post challenge day 1 for each subject
3. To document clinical symptoms from self-reported surveys and standardized symptom scales at baseline and post challenge	a. Computed total symptom score from modified Jackson score through Day 8 and at Day 15. b. Percentage of subjects with total symptom scores that define symptomatic or asymptomatic categories based on modified Jackson score for each subject
Secondary	
1. To assess the safety profile of a live recombinant influenza strain (A/Texas/71/2017 (H3N2), clade 3C3a) following challenge in healthy adult volunteers	a. Number of adverse events (AE) reported from challenge through Day 29 b. Percentage of subjects reporting any AE from challenge through Day 29 c. Number of serious adverse events (SAE) from challenge through Day 57 d. Percentage of subjects reporting an SAE at any time from challenge through Day 57

2. To describe the host serum hemagglutination inhibition and microneutralization antibody responses at baseline and post-challenge	a. Percentage of subjects with serological conversion defined as a minimum 4-fold rise in post-challenge serum antibodies (HAI and MN)[3] to influenza infection at Days 8, 15, and 29, or latest time point available, post challenge compared to baseline (Day -1). b. HAI and MN antibody geometric mean titers (GMTs) at baseline (Day -1) and GMTs and Geometric Mean Fold Rise (GMFR) post-challenge at Days 8, 15, and 29, or latest time point available
3. To describe anti-HA-stalk antibody titer at baseline and post-challenge	a. Percentage of subjects with serological conversion defined as a minimum 4-fold rise in post-challenge HA-stalk specific serum antibody response to HA group 2 stem domains at any day up to Day 29, or latest time point available, post challenge compared to baseline (Day -1). b. For HA-stalk specific response, the GMTs to HA group 2 stem domains at baseline (Day -1), and GMTs and GMFR at approximately Days 8, 15, and 29, or latest time point available.
Exploratory	
1. To describe the host serum neuraminidase inhibiting antibody responses at baseline and post-challenge	a. Percentage of subjects with serological conversion defined as a minimum 4-fold rise in post-challenge serum antibodies (NAI)[4] to influenza infection at Days 8, 15, and 29, or latest time point available, post challenge compared to baseline (Day -1). b. NAI antibody geometric mean titers (GMTs) at baseline (Day -1) and GMTs and Geometric Mean Fold Rise (GMFR) post-challenge at Days 8, 15, and 29, or latest time point available
2. To describe the host immune responses (innate, serum antibody, nasal IgA) at baseline and post-challenge in at least a subset of subjects	a. GMT and GMFR responses for A/Texas/71/2017/H3N2-specific sIgA, non-normalized and normalized for total IgA content, in NLF at baseline (Day -1), and post-challenge Days 8, 15, and 29. b. Percentage of subjects with a minimum of 4-fold increase in nasal IgA content over baseline. c. Levels of A/Texas/71/2017/H3N2 induced blood cytokines and chemokines at baseline (Days -2 and -1), and Days 2-8. d. Levels of A/Texas/71/2017/H3N2 induced cytokines and chemokines in NLF at baseline (Day -1), and post-challenge Days 2-8.
3. To describe physiological responses from an influenza infection as assessed by wearable devices at baseline and post-challenge	a. Physiological data (heart rate variability, temperature, sleep) at baseline and post challenge Days 1-8 associated with influenza infection.

4. To describe systemic transcriptional responses at baseline and post-challenge in at least a subset of subjects	a. Peripheral blood gene expression profiling based on sequencing at baseline (Days -2 and -1) and post-challenge Days 2-8, and 15 in a subset of subjects
5. To compare the clinical features of symptomatic RT-PCR-positive and RT-PCR-negative illness post-challenge	a. Self- and investigator-solicited report of clinical symptoms and their severity as measured by component questions in FLU-PRO and Validation Diary and modified Jackson score by post-challenge Study Day 15 and qualitative RT-PCR positive status
6. To explore alternative case definitions (e.g., MMID) for symptomatic RT-PCR-positive influenza virus infection	a. The combination of symptoms and symptom severity as determined by report using the FLU-PRO and Validation Diary or modified Jackson score which optimize sensitivity and/or specificity for classifying influenza virus infection based on qualitative RT-PCR.
7. To determine the symptomatic background within uninfected participants and between sites	a. Compare symptoms from subjects inoculated with shams to subjects inoculated with virus
8. To evaluate the plasmablast response post-challenge in a subset of study subjects	a. A/Texas/71/2017/H3N2-specific plasmablasts, their specific-antibody (*e.g.*, anti-H3, -N2 and – H3N2 virus) production and isotype (*e.g.*, IgG, IgA) using an enzyme-linked immunospot (ELISpot) assay in PBMCs on Day 8 in a subset of subjects
9. To describe mucosal and systemic human immune cells in blood and NLF and/or nasal curettage at baseline and post-challenge in at least a subset of study subjects	a. The frequency, duration, timing and phenotypic characteristics of immune cells in the blood of subjects at baseline (Day -1) and at approximately on Days 4, 6, 8, and 29 in a subset of subjects b. The frequency, duration, timing and phenotypic characteristics of immune cells in the nasal specimens in subjects at baseline (Day -1) and Days 2, 4, 6 and 8 in a subset of subjects
10. T cell activation in cells from nasal curettage in a subset of study subjects	a. Frequency of activated T cells after challenge (Days -2, 15, 29, and 57) in a subset of subjects
11. To assess the association of human leukocyte antigen (HLA) class I and II alleles with clinical, immune and viral responses in at least a subset of subjects	a. The frequency of HLA class I and II alleles as measured by genetic testing at baseline (Day -1) in a subset of subjects b. Associations of subject HLA class I and II alleles with development of infection post-challenge through Day 8 and magnitude and breadth of the elicited immune responses post-challenge in a subset of subjects
12. To evaluate cellular immune response at baseline and post-challenge in at least a subset of subjects	a. Frequency of influenza-specific B cell subsets in circulation (PBMC) at baseline (Day -1), and at approximately on Days 2, 8, 15, 29, and 57 in a subset of subjects b. Magnitude of influenza-specific T cell responses in circulation (PBMC) to conserved T cell epitopes and inactivated virus at baseline (Day -1), and at approximately on Days 2, 8, 15, 29, and 57 in a subset of subjects

	c. Presence of hemagglutinin (HA)-specific CD4 T cells focusing on Tfh cells on Day 8 on a subset of subjects
13. To describe viral detection by quantitative culture from study subjects at baseline and post-challenge	a. Percentage of subjects within a challenge dose group with detected viral shedding in NLF and/or NP swab(s) each day post-challenge from Day 2 through the end of the confinement period using quantitative viral culture. b. Magnitude of virus shedding post-challenge in each subject defined as the peak viral load in NLF and/or NP swab(s) from Day 2 through the end of the confinement period by quantitative viral culture.

[1] The optimal infectious dose is the minimal infectious dose that is considered safe and maximizes the AR and symptoms among the doses assessed
[2] RT-PCR = reverse transcriptase – polymerase chain reaction
[3] HAI = hemagglutination inhibition; MN = microneutralization
[4] NAI = neuraminidase inhibition

4. STUDY DESIGN

4.1 Overall Design

This is an exploratory Phase 1, blinded, randomized, dose-finding influenza virus challenge study in approximately between 62-114 (targeted maximum sample size of 106 active plus 8 shams) males and non-pregnant females, 18 to 45 years old, inclusive, who are in good health and meet all eligibility criteria. The clinical study is designed to determine the optimal infectious dose and safety profile of a recombinant influenza A/Texas/71/2017 (H3N2) clade 3C3a strain in healthy adult volunteers.

The study will enroll approximately between 13-39 subjects in each of up to 3 challenge dose groups at 2 sites (Duke University [DU] and University of Maryland [UMD]) for approximately between 62-114 subjects total for the study. We plan to enroll each challenge dose group according to an adaptive dose-escalation schedule (**Figure 1**) which allows study results (see Section 9.5.6) to be analyzed between challenge cohorts to inform the sample size and virus dose for the next escalation cohort(s). The study timeline anticipates 4-6 weeks of lead-time for study-specific screening and start-up activities for the initial cohort, and similarly, 4-6 weeks between subsequent cohorts at each site to evaluate study results and turn-over of the clinical unit. However, to compress the overall dosing timelines, challenge cohorts may be performed concurrently or around a 2-week timeline between the sites. We plan up to 2 weeks (Day -2 to Day 8) to complete the clinical in-patient component for each challenge cohort, thus altogether projecting the following to complete the dose-ranging clinical confinement component: up to 3.5 to 4 months, if 3 challenge cohorts evaluated; 4 to 4.5 months, if 4 challenge cohorts evaluated; 5 to 6 months if 5 challenge cohorts evaluated; and 5.5 to 6.5 months if 6 challenge cohorts evaluated. Each participant has an additional 7 weeks of follow-up post confinement. The study period could range from approximately 7.5 months to one year, depending upon the number of challenge cohorts enrolled.

Potentially eligible study subjects may be pre-screened under an active screening protocol (DMID 20-0004) to identify those with HAI antibody titers ≤1:40 specific to the challenge virus strain.

During the study screening period of this protocol (Day -45 to Day -3), participants who have not received a seasonal influenza vaccine within the past 4 months and for whom there are no safety concerns from standard safety assessments and screening labs, are eligible for enrollment to the study. Backup subjects will be confined under respiratory isolation prior to challenge to ensure sufficient number of volunteers undergo challenge, given the potential for dropouts, development of exclusion criterion, and/or pre-challenge evidence of viral respiratory infection among subjects. The number of backup subjects 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. Safety assessments and safety labs include a medical history review, physical examination, ECG, a chest X-ray (CXR), HIV and hepatitis B/C testing, baseline SpO_2 saturation, and testing for WBCs, ALC, hemoglobin, platelets, ALT, and creatinine. Women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test on Day -2. Likewise, participants (including

backup subjects) must have a negative drug urine toxicology test (unless the drug is deemed acceptable by the Investigator), negative multiplex respiratory viral test, and a negative SARS-CoV-2 RT-PCR test at screening and on Day -2. After a standard physical exam to exclude active infection or other illness, participants will begin confinement on Day -2 and remain confined for up to 12 days (Days -2 to 10). Backup subjects will also begin confinement on Day -2 (either within the confinement facility or at an alternate location under respiratory isolation). They will be discharged prior to challenge if they are not needed. All backup subjects replacing discharged subjects will be admitted to the confinement unit prior to viral challenge and remain confined in the confinement unit through Day 10.

On Day 1, subjects will be randomized and administered a single challenge dose of either a sham inoculum or a virus dose between 10^4 and 10^6 $TCID_{50}$. The challenge inoculum will be administered using the MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, Morrisville NC) attached to a 1cc syringe. Approximately 500 µL of study product will be delivered in each nostril with the subject in a recumbent position. Subjects, clinical study staff, administrators of challenge inoculum, and clinical evaluators will be blinded as to whether the participant received either sham or virus challenge inoculum. The dosing plan will follow an adaptive dose-escalation schedule (**Figure 1**) that will include up to 8 different challenge cohorts. This dosing plan is designed to optimize subject safety, starting with a low dose and allowing flexibility to make informed adjustments between challenge cohorts from outcome results of the previous challenge cohort. For the first exposure in humans, the initial low dose challenge will be conducted in a group of a targeted total of 13 subjects (including 1 sham) to assure the virus is safe with no study-related or unanticipated adverse events observed or reported before further use. Dose escalation to the next (higher) dose and subsequent higher doses, if necessary, will be informed by both safety and AR outcomes given a pre-specified decision algorithm (see section 9.3.2) for dose-escalating.

Clinical outcomes and pre-defined halting criteria will be used to determine whether the trial moves forward to the higher dose of the challenge strain.

Clinical Outcomes

Symptomatic influenza virus infection criteria will be based on the virologic assessment and the modified Jackson score. A subject will be defined as having symptomatic influenza virus infection if the following criteria are met:

- Viral shedding detected in NP swab(s) by qualitative RT-PCR on at least two days beginning 24 hours after challenge until Study Day 8.
- A cumulative symptom score ≥ 6 from daily component symptoms, including documented fever, computed across any consecutive 5-day window through Day 8 beginning on Day 2 post challenge using a modified Jackson score. Highest point score per day per sign or symptom will be used. Component signs and symptoms include fever, lymphopenia (< 1000 cells/mL), runny nose, stuffy nose, sneezing, sore throat, headache, cough, malaise, body ache, chills, feverish, shortness of breath, and earache ranked on a 4-point severity scale (0-3). (Appendix A for Temperature). Temperature will be measured three times a day and symptoms will be collected twice daily on the modified Jackson score.

*Note: A documented fever and feeling feverish will count as **one point** on the cumulative symptom score.*

Signs and symptoms will also be collected via the FLU-PRO and Validation Diary instrument which will be completed once daily after 3:00 pm through confinement. Subjects will continue to complete the FLU-PRO daily after discharge through Day 15. The modified Jackson score and FLU-PRO will be compared in exploratory analyses.

- Component signs and symptoms of the FLU-PRO and Validation Diary include runny/dripping nose, congested/stuffy nose, sinus pressure, scratchy or itchy throat, sore or painful throat, difficulty swallowing, teary or watery eyes, sore or painful eyes, eye sensitivity, trouble breathing, chest congestion, chest tightness, dry or hacking cough, wet or loose cough, nausea, stomach ache, dizziness, head congestion, headache, lack of appetite, excessive sleepiness, body aches or pains, weakness or tiredness, chills or shivering, feeling cold, feeling hot, and sweating.

Safety Threshold

The safety threshold is defined as meeting one of the protocol-defined halting criteria outlined in Section 7.1.1 which includes the occurrence of severe influenza complications in any study subject (Section 2.3.1). If a subject has a severe influenza complication(s), the study staff and subject will be unblinded as to receipt of challenge virus or sham and as to the results of influenza RT-PCR testing for safety evaluation and potential therapeutic intervention with an approved influenza antiviral agent.

In this adaptive schema, the initial cohort (Cohort 1A) for the lowest challenge dose group will include a targeted total of 12 subjects receiving 10^4 $TCID_{50}$ of the challenge strain and one subject receiving sham placebo. If fewer than 80% of subjects meet the case definition for influenza in Cohort 1A, the dose will escalate to 10^5 $TCID_{50}$ to include Cohort 2A. If 80% or more subjects in Cohort 1A meet the case definition for influenza, the lowest challenge dose group will be expanded to include a targeted total of 12 additional subjects receiving 10^4 $TCID_{50}$ of the challenge strain and one subject receiving sham placebo to comprise Cohort 1B. Cumulatively, if fewer than 80% of subjects in Cohorts 1A and 1B meet the case definition for influenza, the dose will escalate to 10^5 $TCID_{50}$ to include Cohort 2A. If 80% or more of the subjects in Cohorts 1A and 1B meet the case definition for influenza than the lowest challenge dose group will be expanded to include a targeted total of 12 additional subjects receiving 10^4 $TCID_{50}$ of the challenge strain and one subject receiving sham placebo to comprise Cohort 1C. Cumulatively, if fewer than 80% of subjects in Cohorts 1A, 1B, and 1C meet the case definition for influenza the dose will escalate to 10^5 $TCID_{50}$ to include Cohort 2A. If 80% or more of the subjects in Cohorts 1A, 1B, and 1C combined meet the case definition for influenza then the optimal dose of 10^4 $TCID_{50}$ will be selected.

The 10^5 $TCID_{50}$ challenge dose group similarly includes up to three cohorts each with a targeted total of 12 subjects receiving 10^5 $TCID_{50}$ of the challenge strain and one subject receiving sham placebo with the thresholds for dose escalation to 10^6 $TCID_{50}$ being < 70% subjects for Cohorts 2A, 2B, and 2C cumulatively moving from Cohort 2A to Cohort 2B and Cohort 2C, respectively. The highest challenge dose group to be evaluated in this study is 10^6

$TCID_{50}$, with each of two challenge cohorts including a targeted total of 17 subjects receiving 10^6 $TCID_{50}$ of the challenge strain and one subject receiving sham placebo.

Following virus challenge, subjects will be monitored daily for up to 7 days post-challenge (Days 2-8). During this confinement period, time-series collections of biological samples and clinical information will occur daily from pre-challenge (baseline) through Study Day 8**.** The different specimen types and clinical assessments are shown in **(**Table 1**).** Whole blood samples will be processed into component parts (e.g., PBMCs, plasma, sera, RNA, DNA) to evaluate multiple host response systems including the immune, molecular, and cellular responses in the circulation and nasal mucosa. Additionally, subjects will be provided wearable devices for continuous monitoring of physiological measures including heart rate, ECG, body temperature, and sleep habits.

Examples of the devices used in the study include those listed below. Other devices approved for consumer and/or clinical use may also be used, provided they are fully non-invasive and do not present any additional risk to study subjects:

• Garmin Vivosmart 4: This commercially available product is intended for training and recreational use. It is worn on the wrist and uses photoplethysmography (PPG) technology along with mechanical sensors to measure heart rate, oxygen saturation, activity, etc. The Garmin will transfer the subject's data via Bluetooth to the smartphone where the compatible app has been installed. These apps will then upload the data to cloud-based servers maintained by the individual device manufacturers. Data will then be uploaded to a secure server at the study sites.

• Oura Ring: This commercially available product is intended for training and recreational use. It is worn on the finger and uses photoplethysmography (PPG) technology along with mechanical sensors to measure heart rate, body temperature, activity, etc. The Oura Ring will transfer the subject's data via Bluetooth to the smartphone where the compatible app has been installed. These apps will then upload the data to cloud-based servers maintained by the individual device manufacturers. Data will then be uploaded to a secure server at the study sites.

• Bittium Faros 180: This FDA approved and CE marked medical device is available for clinical use; specifically for outpatient monitoring or occupational therapy after a cardiac event. It has an ECG sensor and accelerometer and can be worn using cable/electrode connections, or over the chest with a fabric strap. Data collected by the Faros 180 will be manually uploaded (via USB) to a secure cloud server maintained by the sites.

For safety assessments, ECG, and safety lab results will be evaluated at pre-specified timepoints as well as daily targeted physical exams during confinement. Viral shedding will be measured by quantitative RT-PCR and culture, as well as qualitative multiplex RT-PCR tests using the BioFire or Luminex respiratory viral panel performed daily on nasal swab(s). In parallel to specimen collections, subjects will provide self-reported symptom diaries once daily through Study Day 15 using a standard symptom instrument, FLU-PRO and Validation Diary. The modified Jackson score will be administered by study investigators twice daily through confinement and once at the Study Day 15 visit. On Study Day 8, subjects may 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 qualitative RT-PCR tests on NP swabs (that are collected 12 hours apart on Study Day 6 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 (< 100.6°F/38.1°C).
- $SpO_2 \geq 95\%$ on room air
- Show no signs or symptoms of moderate or severe influenza (Section 2.3.1) by clinical evaluation.
- Are clinically and hemodynamically stable for at least 48 hours (per the evaluation of the licensed study clinician listed on the Form FDA 1572).
- All AEs and SAEs will be recorded on the appropriate DCF.

Subjects and study staff performing clinical evaluations and assessments will remain blinded to results of RT-PCR testing for influenza only until Study Day 6 or upon early discharge. The study team will not be blinded to RT-PCR testing for other respiratory pathogens. Subjects will be offered a course of an antiviral (oseltamivir or baloxavir marboxil) if they remain symptomatic and/or have a positive influenza test by qualitative RT-PCR on Study Day 6. Treatment can begin as early as Study Day 6 after test results are received. Subjects will remain in confinement until they have two negative influenza tests by qualitative RT-PCR. The two tests must be collected at least 12 hours apart. Subjects will return for three follow-up visits at approximately Days 15, 29 and 57 post-challenge to assess overall health and for additional specimen and clinical data collections.

Schedule of assessments can be found in **Section 1.2, Schedule of Activities**
Protocol Schema can be found in **Section 1.3, Study Schema**.
Dose escalation or dose-ranging details are found in **Section 6.1.2, Dose Escalation**
Full details of interim analysis are found in **Section 9.5.6, Planned Interim and Early Analyses**

4.2 Scientific Rationale for Study Design

Eligible participants will sequentially be enrolled into challenge cohorts and will be randomly assigned to receive a single dose of either sham inoculum or a virus dose between 10^4 to 10^6 $TCID_{50}$. This study uses an adaptive dose-escalation design in order to most efficiently determine the optimal infectious dose with an AR ranging from 55 to 85% depending on the dose and, at the same time, closely monitor subject safety. This adaptive dose-ranging allows adjustments to challenge dose group size and escalation dose schedule to be informed by the AR and safety results. Also, the study design allows for the study to progress across 2 sites with one site lagging behind the other by a period of around two weeks in order to achieve efficiency in enrollment of cohorts. Randomization to challenge virus or sham placebo is essential to facilitate objectivity in symptom reporting. One sham per challenge cohort will be included such that it is a possibility for any study subject to receive sham placebo, and thereby, subjects and evaluators would be more likely to objectively report and rate their self-reported symptoms if it was possible that they could have received sham placebo. Collecting clinical, virologic and immunologic data on enrolled subjects using a standardized timeline and collection instruments should provide valuable information to determine the optimal dose of the challenge strain.

4.3 Justification for Dose

Subjects will be randomized and administered a single challenge dose of either sham placebo or a virus dose between 10^4 to 10^6 $TCID_{50}$. Historically, a 10^4 $TCID_{50}$ has been safely used as the lowest dose tested in challenge studies[5-7]. There is no indication from non-clinical studies of the recombinant influenza A/Texas/71/2017 (H3N2), clade 3C3a virus in mouse, hamster and ferret models of infection that this virus will behave any differently in humans than the wild-type parent influenza virus.

The dosing plan will follow an adaptive dose escalation schedule (**Figure 1**) that will include up to three different challenge dosing groups. This dosing plan is designed to optimize subject safety, starting with a low dose and allowing flexibility to make informed adjustments between challenge cohorts from outcome results of the previous challenge cohort. The plan is guided by our experience and the results of our 3 previous human titration studies.

For the first exposure in humans, the initial low dose challenge will be conducted in a group of a targeted total of 12 subjects (and 1 sham) to assure the virus is safe with no study-related or unanticipated adverse events observed or reported. Dose escalation to the next dose level and subsequent higher doses, if necessary, will be informed by both safety and AR outcomes given a pre-specified decision algorithm for dose-escalating. The decision to escalate or not escalate to the next challenge dose will involve the following criteria:

- The tested dose is determined to be safe with no occurrence of halting criteria or severe influenza complications
- An AR < 80% triggers dose escalation in the 10^4 $TCID_{50}$ challenge dose group.
- An AR < 70% triggers dose escalation in the 10^5 $TCID_{50}$ challenge dose group.
- An AR ≥ 80% in the 10^4 $TCID_{50}$ challenge dose group would stop escalation, and a repeat of the presumed optimal dose for up to 3 total challenge cohorts: 1A, 1B and 1C.
- An AR ≥ 70% in the 10^5 $TCID_{50}$ challenge dose group would stop escalation, and a repeat of the presumed optimal dose for up to 3 total challenge cohorts: 2A, 2B and 2C.

NOTE: AR is the percentage of participants meeting the pre-defined symptomatic influenza infection definition described in section 4.1.

5. STUDY POPULATION

The study population will include male and female adult volunteers aged 18-45 years, inclusive, and generally in good health.

5.1 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 7 days after challenge AND until they have no viral shedding[1], determined by qualitative RT-PCR beginning on Study Day 6

   [1]No viral shedding is defined as two negative RT-PCR tests 12 or more hours apart
4. Healthy[2] males and non-pregnant, non-breastfeeding females aged ≥ 18 and < 46 years of age at enrollment

   [2]Healthy is defined in inclusion criteria #11.

   NOTE: Female subjects of childbearing potential must have a negative serum pregnancy test at screening, a negative urine pregnancy test upon admission to the confinement unit AND a negative pregnancy test before any CXR (if ≥ 7 days have passed since a serum pregnancy test).
5. Women of childbearing potential[3] must agree to use or have practiced true abstinence[4] or use at least one acceptable primary form of contraception[5] for at least 30 days prior to challenge

   [3]Not of childbearing 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).

   [4]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).

   [5]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. Must use at least one acceptable primary form of contraception for at least 30 days prior to challenge and at least one acceptable primary form of contraception during the remainder of the study or approximately 57 days after confinement (Day -2).

NOTE: These criteria are applicable to female subjects in a heterosexual relationship AND of child-bearing potential. These criteria do not apply to subjects in a same sex relationship.

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

   [6]*Non-habitual smokers are those who smoke no more than four cigarettes, other tobacco products, e-cigarettes (to include vaping and Juuling products) or marijuana in a week 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 within the last 2 years and agrees to abstain from alcohol for at least one week before admission and throughout the confinement period

8. No self-reported or known history of restricted drug use for at least 30 days prior to challenge and agrees to abstain from restricted drugs throughout the confinement period

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

   [8]*Select drug use may be allowed at Investigator's discretion (e.g., prescribed amphetamines for ADHD).*

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

    [9]*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)*

11. In good health[10], and do not have clinically significant medical, psychiatric, chronic or intermittent health conditions including those listed in Exclusion Criteria (Section 5.2)

    [10]*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 5.2).*

    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 5.2) 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.

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 ≤ 18
    - Oral temperature is less than 100.6°F

13. Eligibility laboratory values (WBC, Absolute Lymphocyte Count, Hgb, PLTs, ALT and Cr) are within acceptable parameters[11]

    [11]Labs within normal range or grade 1 abnormalities deemed not clinically significant by a study investigator are considered acceptable

14. Body mass index (BMI) >18.5 and <40 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[12]

    [12]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
17. Negative respiratory virus panel by BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or by Luminex xTAG® on Day -2, and Day -1
18. Negative RT-PCR test for SARS-CoV 2 on screening and Day -2
19. HAI antibody titer ≤1:40 against influenza A/Texas/71/2017 (H3N2) at screening
20. Receipt of the recommended number of doses of an EUA authorized or licensed COVID-19 vaccine product ≥ two weeks prior to confinement (Day -2).

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participating in the study:

1. Presence of self-reported or medically documented significant medical or psychiatric condition(s)[1]

   [1]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 [Inhaled, oral or intravenous (IV) corticosteroids, leukotriene modifiers, long and short acting beta agonists, theophylline, ipratropium, biologics] or any treatment for respiratory disease

exacerbations (e.g., asthma exacerbation) within the last 5 years

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

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)

f. Presence of an autoimmune disease.

g. Immunodeficiency of any cause.

h. History of diabetes.

2. Presence of immunosuppression or any medications that may be associated with impaired immune responsiveness[2]

   [2]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 6.5).

3. Known allergy or intolerance to treatments for influenza (including any neuraminidase inhibitors or baloxavir marboxil).

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

5. Known allergy to excipients[3] in the challenge virus inoculum.

   [3] Sucrose, KH_2PO_4, K_2HPO_4, L-glutamic acid (in SPG diluent)

6. Receipt or planned receipt of any investigational drug/investigational vaccine/licensed vaccine, except for a licensed or emergency use authorized COVID-19 vaccine product, within 30 days prior to the date of challenge.

7. Prior enrollment in an influenza virus challenge study with an influenza virus of the same subtype within the past 2 years.

8. Currently enrolled in any investigational study or intends to enroll in such a study within the ensuing study period.[4]

   [4]Co-enrollment in an observational study, an investigational study in a follow-up/post-vaccination stage, or a study involving a licensed drug or biologic may be allowed at the investigator's discretion.

9. Receipt of any influenza vaccine four months prior to challenge or plans to receive influenza vaccine during the study (approximately 57 days after challenge).

10. History of a previous severe allergic reaction to any drug or biologic with generalized

urticaria, angioedema, or anaphylaxis.

11. Receipt of blood or blood products during the six months prior to the planned date of challenge.
12. Plans to donate blood or blood products during the study (approximately 102 days).
13. Any condition (including medical and psychiatric conditions) that, in the opinion of the Investigator, might interfere with the safety of the subject and/or study objectives.
14. An ongoing symptomatic condition for which subject has had or has ongoing medical investigations but has not yet received a diagnosis or treatment plan e.g., ongoing fatigue without a diagnosis.
15. Known close contact with anyone known to have or suspected to have a respiratory viral illness within 7 days prior to challenge.
16. Significant abnormality altering anatomy of nose/nasopharynx (including significant nasal polyps), clinically significant nasal deviation, or nasal/sinus surgery within 180 days prior to challenge.
17. History in the last five years of chronic or frequent intermittent sinusitis.
18. Recent history (180 days) of epistaxis or anatomic or neurologic abnormality impairing the gag reflex or contributing to aspiration.
19. Currently using an internal cardiac device such as a pacemaker or other implanted electronic medical devices.

5.2.1 Exclusion of Specific Populations

For safety purposes, we are targeting a young, healthy population. We are excluding persons over 45 years of age and children and adolescents under 18 years of age. We are also excluding pregnant women and women who are planning to become pregnant from 30 days prior to challenge through the end of the study.

5.3 Inclusion of Vulnerable Participants

Not applicable.

5.4 Lifestyle Considerations

During this study, participants are asked to:

- Remain in a confinement unit for a minimum of 7 days following challenge (total of 10 days) or until they have at least 2 consecutive negative influenza tests performed 12 hours apart starting on Study Day 6 of the study.
- Refrain from drinking alcohol or restricted drug use for one week prior to confinement and during the confinement period.

- Refrain from taking any forbidden medications including over-the-counter medications (including non-steroidal anti-inflammatory medications) for one week prior to confinement and during the confinement period (unless approved during confinement).
- Refrain from enrolling in any other investigational study throughout the duration of the study.
- Refrain from donating blood or blood products throughout the duration of the study and for 2 months prior to challenge.
- For women of childbearing potential, avoid getting pregnant and use contraception within 30 days from challenge.

5.5 Screen Failures

After the screening evaluations have been completed, the investigator or designee will review the inclusion/exclusion criteria and determine the subject's eligibility for study randomization.

Subjects who are found to be ineligible will be told the reason for ineligibility and will be replaced. Individuals who do not meet the criteria for participation in this study (screen failure) because of a current respiratory illness may be re-screened after 7 days.

5.6 Strategies for Recruitment and Retention

5.6.1 Recruitment

Potential participants may be selected from the existing cohort that were pre-screened for influenza antibody titers in the screening protocol DMID 20-0004 and from other existing participant registries, through advertising or by word of mouth. Potentially eligible participants for this study must consent to the repository protocol, DMID 19-0025, prior to initiation of any screening activities. Recruitment staff at all sites will contact those individuals enrolled in 20-0004 and 19-0025 to assess their eligibility for this study. It is anticipated that the demographic composition of the 20-0004 cohort and subsequent subjects for this study will be representative of the respective study site region.

The IRB will approve the recruitment process and all materials provided prior to any recruitment to prospective subjects directly.

Screening will begin with a brief discussion with study staff. Some people will be excluded based on demographic data and medical history (i.e., pregnant, recently vaccinated, etc.). Information about the study will be presented to potential subjects and questions will be asked to determine potential eligibility.

5.6.2 Retention

Retention of subjects in this trial is very important for determining the primary endpoint. As such, after discharge from confinement, participating subjects will be reminded of subsequent study visits and every effort will be made to accommodate the subject's schedule to facilitate follow-up within the specified visit window. Additionally, there are many circumstances that influence the ability to obtain outcome information after discharge. Follow-up visits may be conducted by phone if in person visits are not feasible. Lastly, compensation strategies will be implemented to encourage subjects to return for follow-up assessments and sample collections.

5.6.3 Compensation Plan for Subjects

Compensation will be determined locally and in accordance with IRB requirements, and subject to IRB approval. Compensation will be described in the site-specific informed consent.

5.6.4 Costs

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. However, procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party.

6. STUDY PRODUCT

6.1 Clinical Challenge Strain

A GMP lot of reverse-genetics (RG) derived, recombinant influenza A/Texas/71/2017 (H3N2) virus for the purpose of conducting human influenza virus challenge studies has been manufactured by Charles River Laboratories (CRL), Malvern, PA. As part of the GMP manufacturing process, a number of tests have been performed to assure the virus is genotypically and phenotypically accurate, including genomic sequencing of the GMP-grade virus. In addition, pre-clinical animal testing has been performed in ferrets, mice, and hamsters to demonstrate that manufactured virus behaves similarly in animals to the wild-type strain from which it was derived. Non-clinical testing results are included in the Investigator's Brochure (IB) for the Live Influenza Virus RG-A/Texas/71/2017 (H3N2).

The challenge virus is nearly identical to circulating influenza A/Texas/71/2017 (H3N2) viruses that are susceptible to FDA-approved neuraminidase inhibitors oseltamivir and zanamivir. Full genomic sequencing was performed on the Live Influenza Virus RG-A/Texas/71/2017 (H3N2) (Lot1507-232149) and did not indicate any mutations that confer resistance to these antivirals. The 2017 H3N2 viruses are resistant to the amantadine class of influenza antivirals [8]. There is no indication that the Live Influenza Virus RG-A/Texas/71/2017 (H3N2) challenge virus would behave any differently in humans than the wild-type influenza A/Texas/71/2017 (H3N2) virus, based on the available *in vitro* and *in vivo* data.

The diluent for the challenge strain is 1X SPG (7.4% sucrose, 3.8 mM KH_2PO_4, 7.2 mM K_2HPO_4, 5.4 mM L-glutamic acid), Lot #141701, produced using GMP manufacturing processes at CRL, Malvern, PA.

6.1.1 Dosing and Administration

Table 2. Dosing and Administration

Product Name	Doses	Route	Frequency of Administration
Live Influenza Virus RG-A/Texas/71/2017 H3N2	1.0 mL (0.5 mL per nostril) of approximately 10^4, 10^5, or 10^6 $TCID_{50}$ each dose *	Intranasal by atomizer delivery	Challenge virus will be administered once on Day 1
Sham (SPG) inoculum	1.0 mL (0.5 mL per nostril) of vehicle inoculum	Intranasal by atomizer delivery	Inoculum will be administered once on Day 1

* $TCID_{50}$, median tissue culture infectious dose

6.1.2 Dose Escalation

The dose escalation schema is provided in **Figure 1.**

6.1.3 Dose Modifications

There will be no individualized dose modifications of virus inoculum.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Clinical challenge virus stock and SPG diluent will be sourced by DMID Clinical Material Services (CMS).

Records will be maintained that document receipt, release for dosing, disposal, or return to the sponsor.

Upon request by DMID study products will be transferred to the following address:

DMID Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

Accountability: 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. Refer to the protocol-specific MOP for details on storing study medications.

Unused study product will be retained until study conclusion or until accountability via verification of inventory or monitoring has occurred and written notification stating retention is no longer required is received as applicable per the site's SOPs. Used study product will be disposed of per the MOP. Documentation of study product destruction will be available to monitors upon request.

Accountability may be performed through verification of inventory and during monitoring. DMID does not require used containers of study product to be maintained at the research pharmacy until the clinical monitors have confirmed the disposition of all study products. Retention of used study product containers for monitoring is only required when the local institution's SOP/policy mandates it. If local SOPs allow/require destruction of used study product containers, then the used vials can be destroyed per the site's SOPs with a second staff member's observation and signed verification (two signatures) that the used vials were destroyed.

Refer to the protocol-specific MOP for details should any vials need retained for viral titer testing.

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

6.2.2 Formulation, Packaging, and Labeling

Live Influenza Virus RG-A/Texas/71/2017 (H3N2)

The final product was vialed at approximately 1.86 X 10^6 $TCID_{50}$/mL of Influenza A Human Challenge Virus (H3N2) in 1X SPG (7.4% sucrose, 3.8 mM KH_2PO_4, 7.2 mM K_2HPO_4, 5.4 mM L-glutamic acid). Each vial has a fill volume of 0.6 mL.

Each vial was individually labeled with the product name, description, and caution statement. The label was affixed to 2 mL cryogenic vials prior to final fill with Live Influenza Virus RG-A/Texas/71/2017 (H3N2) Virus [Charles River Laboratories, Malvern PA] (Lot #1507-232149).

Diluent and Sham Product

1X SPG (7.4% sucrose, 3.8 mM KH_2PO_4, 7.2 mM K_2HPO_4, 5.4 mM L-glutamic acid). Each vial has a fill volume of 1.2 mL. The diluent will also be used as a sham inoculum. Each vial was individually labeled with the product name, description, and caution statement. The label was affixed to 2 mL cryogenic vials prior to final fill with 1X SPG [Charles River Laboratories, Malvern PA] (Lot #141701).

6.2.3 Product Storage and Stability

Live Influenza Virus RG-A/Texas/71/2017 (H3N2)

The human challenge virus will be shipped to the sites and stored at ≤ -65˚C in single-use vials. Additional details of product storage and stability can be found in the MOP.

Diluent/Sham Product

The 1X SPG will be shipped to the sites and stored at -20°C ± 10°C in single-use vials. Additional details of product storage and stability can be found in the MOP.

MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, Morrisville NC)

The MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, Morrisville NC) will be stored at room temperature (15-30°C).

6.2.4 Preparation and Administration

Live Influenza Virus RG-A/Texas/71/2017 (H3N2)

The Live Influenza Virus RG-A/Texas/71/2017 (H3N2) is an infectious influenza virus and requires handling at Biosafety Level 2. All steps in preparing the syringes should be performed with aseptic technique in a biosafety cabinet.

The challenge virus will be administered intranasally, 0.5 mL in each nostril of a recumbent participant using the MAD Nasal™ Intranasal Mucosal Atomization Device attached to a 1 mL

syringe. Detailed instructions for challenge virus inoculum preparation and administration are provided in the MOP.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Study Product Assignment Procedures

Subjects will be randomized to receive either Live Influenza Virus RG-A/Texas/71/2017 (H3N2) challenge virus or sham placebo within each challenge dose group. Subject will receive one of three doses of challenge virus depending upon the challenge cohort into which they enroll using the adaptive trial design. The ratio of subjects randomized to receive either challenge virus or sham placebo is described below.

Challenge Cohort	Challenge Dose Group	Challenge virus:Sham placebo
1A, 1B, 1C	10^4 $TCID_{50}$	Targeted 12:1
2A, 2B, 2C	10^5 $TCID_{50}$	Targeted 12:1
3A, 3B	10^6 $TCID_{50}$	Targeted 17:1

6.3.2 Randomization and Blinding

Eligible subjects for each challenge cohort will be randomized to receive either challenge virus or sham. Enrollment into the active challenge cohort will be completed before the next challenge cohort is opened to enrollment. The subsequent challenge cohort will be selected and opened for enrollment after safety review, as shown in **Figure 1**. To compress the overall dosing timelines, challenge cohorts may be split between the 2 sites. Subjects that drop out less than 48 hours prior to challenge will not be replaced, but additional randomization slots will be included (at the same targeted allocation ratios for each cohort) to allow for the possibility of enrollment beyond the targeted cohort sizes.

The list of randomized treatment assignments will be prepared by statisticians at The Emmes Company, LLC. and included in the enrollment module of Emmes' Internet Data Entry System (IDES). IDES will assign each subject a treatment code from the list after demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided in the MOP for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

6.3.3 Blinding and Masking Procedures

Study product will be prepared by an unblinded pharmacist but administered by a blinded administrator. The subjects, the study personnel who perform study assessments after administration, data entry personnel at the sites, and laboratory personnel performing immunologic assays will be blinded to treatment assignment. The SMC will receive data in aggregate by cohort and challenge dose group, and they may be unblinded to individual study

product assignments, as needed, to adequately assess safety issues. Refer to the MOP for unblinding procedures.

6.4 Study Intervention Compliance

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 on the Treatment Administration Record and entered into the eCRF.

6.5 Concomitant Therapy

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 challenge. Concomitant medications will be reviewed at every study visit through the end of the study. Women of childbearing 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 2019/2020 and/or 2020/2021 influenza vaccine seasons, 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 previous two season's vaccination will be documented. Prior participation in any influenza challenge study will be documented. Note that prior enrollment in any influenza challenge study within the prior two years and/or receipt of any influenza vaccine four months prior to challenge is exclusionary.

The following prescription or over-the-counter medications cannot be used within 7 days prior to admission to and through the confinement period, 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 challenge, with the exception of an EUA authorized or licensed COVID-19 vaccine product ≥ two weeks prior to admission.

Subjects should not have received influenza vaccine four months prior to challenge and must not plan to receive an influenza vaccine during the study period (approximately 57 days).

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

6.5.1 Non-Research Standard of Care

Oseltamivir phosphate (TAMIFLU®) or baloxavir marboxil (XOFLUZA™) will be offered to any subject who has not had two consecutive days of negative qualitative RT-PCR tests for influenza virus by Study Day 8. Oseltamivir is a neuraminidase inhibitor indicated for the treatment of acute uncomplicated influenza A and B in patients 2 weeks of age and older who have been symptomatic for no more than 2 days, and for prophylaxis of influenza in patients 1 year and older. Baloxavir is a polymerase acidic (PA) endonuclease inhibitor indicated for the treatment of acute uncomplicated influenza in patients 12 years and older who have been symptomatic for no more than 48 hours.

Subjects who continue to shed virus beyond Day 8 will undergo routine daily evaluations as described in Sections 5 and 8. The licensed study clinician listed on the Form FDA 1572 may also 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. Escalation of care may result from the development of serious related or unrelated signs or symptoms during confinement period.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Halting Criteria and Discontinuation of Study Intervention

7.1.1 Study Halting Criteria

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

1. Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within one day after challenge.
2. Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within three days after challenge.
3. Any subject experiences a related SAE during the confinement unit period.
    - This includes any subject who develops a severe influenza complication (Section 2.3.1) during the confinement unit period and requires admission to an Intensive Care Unit (ICU), and/or receipt of ICU level of care
4. Two or more subjects per challenge cohort (or four or more subjects per dosing group) experience the same related grade 3 AE of any kind after challenge through the confinement unit period and related to study product.
5. Any medically significant safety issue that the PI determines should halt the study.

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.

7.2 Participant Withdrawal from the Study and Replacement

Participants are free to withdraw from participation in the study at any time upon request. 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 confinement unit before challenge. The investigator should be explicit regarding study follow-up (*e.g.*, safety follow-up) that might be carried out once the subject undergoes challenge but decides to leave the confinement unit before discharge criteria are met.

Subjects will be strongly discouraged from withdrawing from the study post-challenge. Any subjects who withdraw 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, and that they will stay sequestered in their homes and avoid contact with others until they have two consecutive negative tests for influenza virus by qualitative RT-PCR conducted at least 12 hours apart. 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 or a 5-day course of oseltamivir 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 confinement unit prior to Study 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, and 11). 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 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).

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Subject becomes pregnant.
- Study non-compliance that, in the opinion of the investigator, poses an increased risk (i.e., missing safety labs), or compromises the validity of the data.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Any subject that experiences a related SAE during the confinement unit period.
    - This includes any subject who develops a severe influenza complication (Section 2.3.1) during the confinement unit period and requires admission to an Intensive Care Unit (ICU), and/or receipt of ICU level of care
- If the participant met an exclusion criterion for participation in the study (either newly developed or not previously recognized) that precludes further study participation
- 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 becomes lost to follow-up.

If the subject agrees, every attempt will be made to follow all AEs through resolution.

Subjects who withdraw, or 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, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before randomization may be replaced.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening and Outcome Procedures

8.1.1 Screening Procedures

Potentially eligible study subjects may be pre-screened under an active screening protocol (DMID 20-0004) to identify those with HAI antibody titers ≤1:40 specific to the challenge virus strain. Additionally, potentially eligible subjects may be recruited through other site registries, advertisements or by word-of-mouth. During the study screening period (Day -45 to Day -3), participants who meet all inclusion criteria and none of the exclusion criteria are eligible for enrollment to the study. Participants will be screened during the screening period and again at the time of confinement (Day -2).

After the informed consent, the following assessments are performed to determine eligibility and obtain baseline data:

- Take a focused medical history, including the following information:
    - History of any chronic medical conditions related to inclusion and exclusion criteria
    - Review of receipt of 2020/2021 influenza vaccine and 2021/2022 influenza vaccine (note that receipt of 2020/2021 influenza vaccine and receipt of 2021/2022 influenza vaccine within the past 4 months or within approximately 57 days post-challenge is exclusionary)
    - Concomitant medications taken within 90 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility
- Women of childbearing potential must have a negative serum HCG pregnancy test at screening and a negative urine HCG pregnancy test on Day -2.
- Participants must have a negative drug urine toxicology test (amphetamines, cocaine, and opiates) at screening and on Day -2 (amphetamines, cocaine, and opiates), unless the drug is deemed acceptable by the Investigator.
- Physical exam (standard exam at screening and targeted exam on Day -2)
- Height measurement
- Weight measurement
- Vital signs measurement (including RR, HR, BP, oral temperature)
- SpO_2 measurement
- ECG
- Baseline posterior anterior (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.)
- Blood for laboratory evaluations (approximately 15 mL at screening only):
    - Serology at screening: HIV, Hepatitis B surface antigen, Hepatitis C antibody,
    - Chemistry at screening: alanine transaminase (ALT) and creatinine (Cr)
    - Hematology at screening: white blood cells (WBCs), absolute lymphocyte count, hemoglobin, and platelets

- Blood for HAI titer
- Participants must have a negative qualitative multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) including a test for SARS-CoV-2 on NP swab on Day -2 in order to be eligible for influenza challenge
- Nasopharyngeal swab for virologic assessments

Clinical screening laboratory evaluations and the HAI titer will be performed locally by the site laboratory. The volume of venous blood to be collected is presented in **Appendix C.**

The overall eligibility of the subject to participate in the study will be assessed once all screening values are available.

8.1.2 Discharge from Quarantine

Subjects will be discharged from quarantine on or after Study Day 8 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). Subjects who do not meet discharge criteria on Study Day 8 will remain in the confinement unit until the criteria are met. Study procedures for the confinement unit will be the same as on Study Day 8, except no additional blood draws unless clinically indicated.

- Have two consecutive qualitative RT-PCR tests on NP swabs (collected 12 hours apart on Study Day 6 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 (< 100.6°F/38.1°C).
- $SpO_2 \geq 95\%$ on room air
- Show no moderate or severe influenza signs or symptoms by clinical evaluation.
- Are clinically stable for at least 48 hours (per the evaluation of the licensed study clinician listed of the Form FDA 1572).
- All AEs and SAEs will be recorded on the appropriate DCF.

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

If a physiologic parameter, e.g., vital signs, is outside of the protocol-specified range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is the result of an acute, short-term, rapidly reversible condition (e.g., stress, anxiety, or “white coat syndrome”). A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by malfunctioning, or an inappropriate measuring device (i.e., inappropriate-sized blood pressure cuff).

A subject may be re-screened if there is a transient disease status (e.g., subject complained of a "cold or fever" and met a temporary delaying enrollment criterion of acute illness or fever), or if a protocol eligibility criterion that is not met at the initial time of screening, will be met by re-screening at a later date (e.g., a medication taken within exclusionary window at the time of first screening that would not be within exclusionary window at a later rescreen).

No subject may be screened more than twice for the same challenge cohort due to a screening failure result as defined above. However, subjects that screen fail for one challenge cohort can be screened in the future for another challenge cohort.

Participants will be provided the results of any abnormal clinical laboratory results and will be referred to their primary healthcare provider. Research laboratory results will not be provided to the participant.

8.1.4 Outcome / Immunogenicity / Genetic Assessments

The specific timing of procedures/evaluations to be done at each study visit are captured in Section 1.2, Schedule of Activities (SoA). All labs in this section will be completed in research labs, thus not requiring CLIA certification. Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP. Specimen shipment to designated research laboratories for outcome/immunogenicity/genetic assessments 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 protocol-specific MOP.

8.1.4.1 Outcome Evaluations

Clinical assessments

- Symptomatic influenza infection for determining AR will be assessed using a Modified Jackson score collected by study staff twice a day (approximately 8 AM and after 3 PM) from Day -2 until the time of discharge. The investigator will also complete the Modified Jackson Score at the Day 15 visit to assess any ongoing symptoms. The Modified Jackson score will be calculated after evaluation of vital signs, laboratory results, and the Modified Jackson score across multiple days. The score will be calculated during analysis for safety reporting by the SDMCC.

- An additional validated, patient-reported outcome (PRO) measure to standardize assessment of influenza symptoms in clinical research (i.e., FLU-PRO Survey Instrument and Validation Diary) will be completed by subjects once a day (after 3:00 PM) from Day -2 through Day 15. The investigators will review the FLU-PRO and Validation Diary at the Day 15 visit to assess any ongoing symptoms.

- Wearable devices
    - The Garmin uses PPG technology along with mechanical sensors to measure heart rate, oxygen saturation, activity, etc.
    - The Oura Ring uses PPG technology along with mechanical sensors to measure heart rate, body temperature, activity, etc.

- The Faros device has an ECG sensor and accelerometer and can be worn using cable/electrode connections.

Virologic assessments
Virologic assessments will be done at screening (Days -45 to -3), baseline (Days -2 and -1) and will be repeated daily from Days 2-8 during the confinement period or for a longer period if still RT-PCR positive for influenza on Days 7 or 8 until two negative swabs are confirmed, at least 12 hours apart, consecutively. Only qualitative RT-PCR will be performed at the screening visit.

- Qualitative multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®) on NP swab(s).
- Quantitative RT-PCR for influenza from NP swab(s) specimen.
- Qualitative and quantitative RT-PCR for influenza from NLF specimens may be performed.
- Quantitative viral culture from NP swab(s) and/or NLF specimens may be performed.

8.1.4.2 Immunogenicity Assessments

- HAI, MN, NAI, anti-HA-stalk antibody titers from serum
- sIgA titers from NLF
- Systemic cytokines and chemokines from serum
- Mucosal cytokines and chemokines from NLF or nasal curettage specimens
- Transcriptional responses from whole blood
- Plasmablasts (ASC) in circulation (from PBMC)
- Influenza-specific B and T cell immune responses from PBMCs
- T cell activation may be performed in cells isolated from nasal curettages
- Immunophenotyping of innate, B and T cell subsets in NLF or nasal curettage specimens

Please see Appendix C for venipuncture volumes.

8.1.4.3 Genetic/Genomic Analyses

- Human leukocyte antigen (HLA) class I and II alleles from blood
- Transcriptional responses from whole blood

Please see Appendix C for venipuncture volumes.

Genetic Privacy and Confidentiality

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 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.

Management of Results and Genetic Counseling

Subjects will be contacted if a clinically actionable gene variant is discovered either as part of this protocol or a related sub-study. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come back to have genetic education and counseling to explain this result. If the subject does not want to come back, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

8.2 Safety and Other Assessments

Study procedures are specified in the SoA. A study clinician licensed to make medical diagnoses and listed on the 1572 will be responsible for all trial-related medical decisions. Specimens for clinical screening and safety laboratory evaluations will be transported from the participating clinic sites or confinement units to the respective site's certified clinical laboratories or on-site certified laboratories for analysis.

Physical Examination
A physical exam will be conducted at screening (Day -45 to -3) by a licensed study clinician listed on the Form FDA 1572. A more targeted physical examination will be conducted at pre-challenge screen (Day -2), pre-challenge baseline (Day -1), viral challenge (Days 1-8), and follow-up (Days 15, 29, 57). The targeted physical exam will include evaluation of the oral/pharyngeal, neck, lung, and heart exams. An otoscopic exam will be performed as part of the targeted physical exam at baseline (Day -1) and as needed. Additional clinical evaluation will be performed if clinically indicated.

Vital Signs
Vital signs will be assessed at screening (Day -45 to -3), pre-challenge screen (Day -2), pre-challenge baseline (Day -1), pre-challenge baseline (Day 1 prior to challenge), post-challenge (Days 1-8) approximately within each 8-hour period (i.e., three times per day), and follow-up (Days 15, 29, 57). Vital signs will include pulse, systolic blood pressure, diastolic blood pressure, respiratory rate, and oral temperature.

SpO_2
SpO_2will be assessed at screening (Day -45 to -3), pre-challenge screen (Day -2), pre-challenge baseline (Day -1), pre-challenge baseline (Day 1 prior to challenge), post-challenge (Days 1-8) approximately within each 8-hour period (i.e., three times per day) and follow-up (Days 15, 29, 57).

Electrocardiograms (ECGs)
A 12-lead ECG will be performed at screening (Day -45 to -3), post-challenge (Day 6), and as clinically indicated and confirmed by the PI or designated clinician licensed to make medical diagnoses and listed on Form FDA 1572. Screening ECGs must be within normal reference ranges or deemed not clinically significant.

Chest X-Ray (CXR)
A PA and lateral CXR will be performed at screening (Day -45 to -3).

Clinical Laboratory Evaluations

- Serology at screening: HIV, Hepatitis B surface antigen, Hepatitis C antibody, and HAI titer
- Hematology (at screening and study Days 2, 4, and 8): white blood cells (WBCs), absolute lymphocyte count, hemoglobin, and platelets
- Chemistry at (as screening and study Days 2, 4, and 8): alanine transaminase (ALT) and creatinine (Cr)
- A urine toxicology test (amphetamines, cocaine, and opiates,) at screening and on the day of admission to the confinement unit (amphetamines, cocaine, and opiates)
- Serum HCG pregnancy test at screening
- 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 Day -2, to be performed locally.
- Qualitative multiplex respiratory virus assay (BIOFIRE® FILMARRAY® respiratory panel by bioMérieux or Luminex xTAG®), including SARS-CoV-2 RT-PCR, on NP swab(s) at screening and on Day -2 and Day -1 in order to remain on the unit for challenge and daily from Day 2 through the confinement period.

8.2.1 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 Section 5.1 and 5.2, and the protocol-specific MOP.

Signs, symptoms, laboratory findings that are part of mild to moderate influenza disease collected as part of the Modified Jackson Score, in addition to mild or moderate fever and lymphopenia from the time of challenge through 7 days post-challenge, will not be considered adverse events (AEs). These signs, symptoms, and laboratory findings (mild and moderate) include the following: runny nose, stuffy nose, sneezing, sore throat, headache, cough, malaise (tiredness), body aches, chills, feverish, shortness of breath, earache, fever, and lymphopenia. Mild to moderate fever is defined in Appendix A and mild to moderate lymphopenia is defined in Appendix B. Influenza infection related symptoms that are deemed by the investigator to be severe, in addition to severe fever as defined in Appendix A and severe lymphopenia as defined in Appendix B, will be considered as adverse events. Symptoms as assessed by the FLU-PRO will not be assessed as adverse events unless deemed by the investigator to be severe.

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 2 months post-challenge).

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

3. Adverse Events – non-serious adverse events occurring from the time of challenge through approximately 28 days post-challenge. See below for how adverse events are defined.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

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 (21 CFR 312.32 (a)).

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 symptomatic influenza infection will be collected from the time of challenge through 7 days post-challenge and will **not** be considered adverse events. Objective clinical examination findings consistent with the solicited symptom, such as oropharyngeal erythema or lymphadenopathy, will also **not** be considered AEs. Any influenza signs, symptoms or lab findings determined by the clinician to have exceeded the expected severity or any moderate or severe complications, as listed in Section 2.3.1, 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. Events that occur after administration of antivirals will also be considered unsolicited AEs. 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 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.

8.3.1.1 Classification of an Adverse Event

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

8.3.2 Definition of Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes (21 CFR 312.32 (a)):

- death
- a life-threatening adverse event
- 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 hospitalization 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 emergency room or at home, convulsions that do not result in inpatient hospitalization, etc.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study clinician 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 clinician (for investigational new drug application [IND] studies, a licensed clinician 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.

8.3.2.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is any SAE where a causal relationship with the study product is at least a reasonably possible but is not listed in the Investigator Brochure, Package Insert or Summary of Product Characteristics.

8.3.2.2 Severity of Event

All AEs or SAEs will be assessed for severity, according to the toxicity grading scales in Appendices A and B.

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affect clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

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.

8.3.2.3 Relationship to Study Intervention

The licensed study clinician'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 challenge, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3 Time Period and Frequency for Event Assessment and Follow-Up

In this study, solicited symptoms of influenza infection will be collected from the time of challenge through 7 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 a mild or moderate influenza symptom or sign 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. Unsolicited AEs will be captured starting at the time of challenge through 28 days post-challenge. 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 5.1) 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.

8.3.4 Adverse Event Reporting

8.3.4.1 Investigators Reporting of AEs

Information on all AEs should be recorded on the eCRF. All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality. Each AE will also be described in terms of duration (start and stop date), severity, association with the study product, action(s) taken, and outcome.

8.3.5 Serious Adverse Event Reporting

8.3.5.1 Investigators Reporting of SAEs

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 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 appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

8.3.5.2 Regulatory Reporting of SAEs

Following notification from the site Principal Investigator or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse event (SUSAR) as an IND safety report to the FDA and will notify all participating site Principal Investigators (i.e., all Principal Investigators to whom the sponsor is providing drug under its IND(s) or under any Principal Investigator'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 7 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.

SAEs that are not SUSARs will be reported to the FDA at least annually in a summary format which includes all SAEs.

8.3.6 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage electronic data capture system (Advantage eClinical) on the Pregnancy Report form. 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.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Department of Health and Human Services Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Statistical and Data Management Coordinating Center (SDMCC)/study Sponsor and the lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and as per Section 8.3.5 Serious Adverse Event Reporting within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within three days of the investigator becoming aware of the problem.

9. STATISTICAL CONSIDERATIONS

9.1 Introduction

The goal of this study is to find a challenge virus dose that is safe and can achieve a symptomatic influenza AR that will be sufficiently high for utilization in future vaccine or intervention studies. The optimal dose of the three considered is broadly defined as the minimum challenge virus dose that elicits the highest AR without meeting safety-stopping criteria. Additionally, viral recovery, clinical symptoms, and immune responses over the post-challenge period will be described by challenge dose group.

9.2 Statistical Hypotheses

The study is not designed to achieve predetermined levels of power or precision to address the primary, secondary, or exploratory objectives. It is of interest to utilize more of the available sample size at higher doses, unless there is adequate evidence that one of the lower doses is sufficiently infectious. This is further described in Section 9.3.2. General methodology planned to address study objectives is described in Section 9.5, and the specific null and alternative hypotheses and details about planned tables, figures, and listings will be given in a separate statistical analysis plan (SAP).

9.3 Sample Size Determination

9.3.1 Study Design

This is a challenge study of A/Texas/71/2017 (H3N2), clade 3C3a virus infection to assess viral dynamics, clinical and immunological response, and safety. A targeted maximum of114 (targeting 106 active plus 8 shams) participants will be enrolled at the clinical sites and will receive one of 3 doses (10^4, 10^5, or 10^6 $TCID_{50}$) of A/Texas/71/2017 (H3N2), clade 3C3a virus or sham placebo. There will be a targeted maximum of 36 active subjects enrolled into each challenge dose group. For each challenge cohort, a target of 1 sham placebo subject will also be included, with the primary intent of reducing the rate of false positive symptoms due to subjects not being certain whether they are receiving active virus.

Initially, a targeted total of 12 subjects will be enrolled to receive a dose of 10^4 $TCID_{50}$, as well as 1 sham placebo subject. While it is unexpected that this dose would be highly infective, this initial cohort will allow for a safety assessment of the virus and study challenge procedures. The criteria for determining the dose received by the subsequent cohort will then depend on the percentage of those initial subjects meeting the symptomatic influenza criteria, and whether the study stopping-safety criteria have been met. The doses received by each subsequent challenge cohort will be determined in a similar manner, as depicted in **Figure 1**. At the highest dose, both challenge cohorts would include a target of 17 active subjects and 1 sham, in order to increase the total sample size at what are assumed to be doses more likely to meet the criteria for optimality. Escalation will stop when the maximum sample size is reached.

9.3.2 Dose Escalation and Sample Size Allocation

The study is planned to target enrolling a maximum of 114 subjects (targeting 106 active plus 8 shams), at one of 3 challenge virus doses or sham placebo. While the study is not designed to test any specific null hypothesis or reach a pre-determined level of estimation precision, this section gives the precision for estimates and power for hypothesis tests of interest, 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 are given in Section 9.5.1 and will be described further in the SAP.

A targeted total of 13 participants (12 active plus 1 sham) will be planned to enroll for each cohort at the two lowest dose levels, and a targeted total of 18 participants (17 active plus 1 sham) per cohort for the highest dose level. Additional randomization slots will be included to allow for the possibility of enrolling beyond the targeted cohort sizes. These calculations allow for 2 subjects per cohort (of the targeted sizes) to withdraw prior to challenge, and if that does not happen, or if more than the targeted number of subjects are enrolled in a given cohort or dose group, the power and precision would increase (detectable difference would decrease) compared to what is presented in this section. Correspondingly, if more than 2 subjects withdraw the power and precision would decrease.

The dose escalation decision rules have been constructed with the intent of safely escalating doses until there is compelling evidence of a sufficiently high AR at a given dose. Since it is assumed that the AR will increase with the dose, the symptomatic influenza thresholds for dose escalation from one cohort to the next were chosen to increase the expected sample size at the higher doses and improve the precision for estimating the true AR for those doses.

Figure 2 shows the probability of meeting the infection threshold for further enrollment at a dose, given varying numbers of subjects enrolled at that dose (10 enrolled in Cohorts 1A or 2A, 15 at Cohort 3A, or 20 in Cohorts 1A+1B or 2A+2B), varying true infection probabilities, and the thresholds for further enrollment.

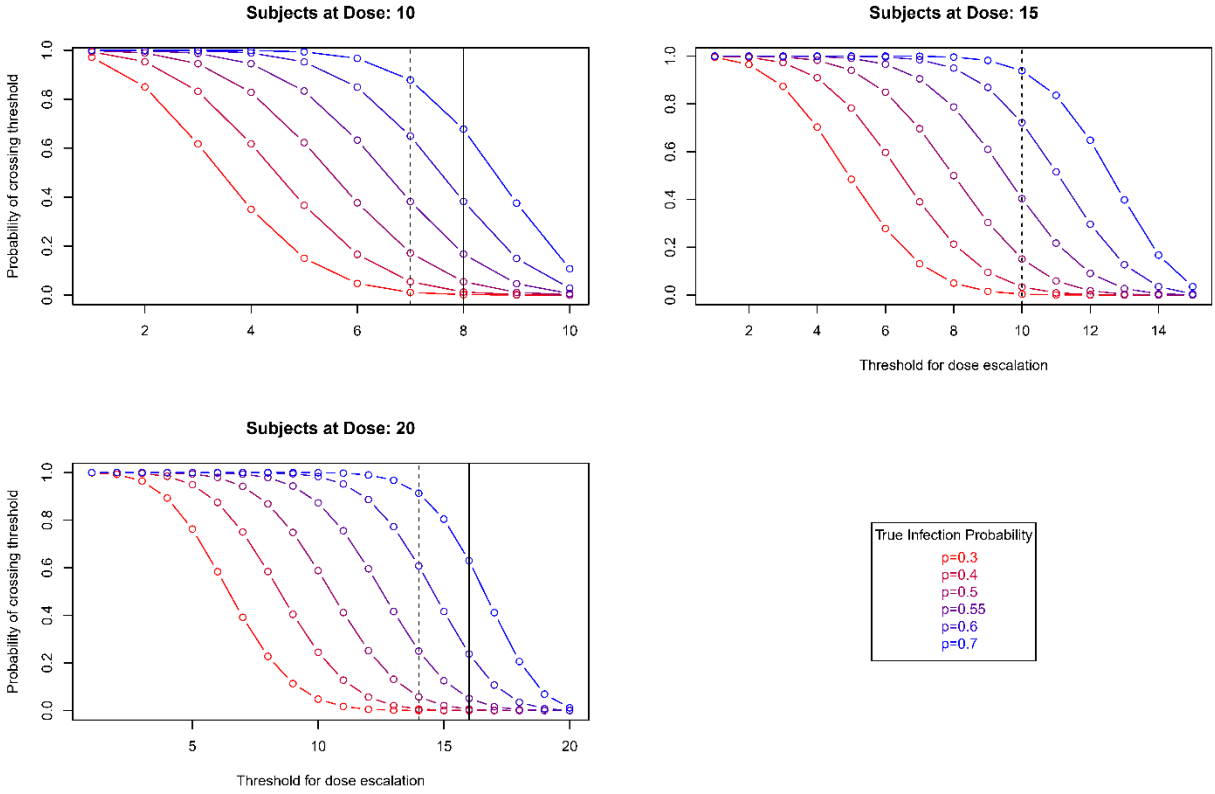

Figure 2. Probabilities of further enrollment at the current dose, given varying true infection probabilities, numbers of subjects enrolled at the current dose, and decision thresholds

For illustration, with n=10 subjects enrolled in Cohort 1A at 10^4 $TCID_{50}$, the probability of achieving 8 infections (solid line, top left panel) would be only 0.01 if the true infection probability were 0.4, compared to 0.67 if the true infection probability were 0.8.

Depending on the number of infections observed at each dose level, the final sample size for each dose group will be approximately 10, 15, 20 or 30. **Figure 3** shows the estimated infection probabilities and associated exact 95% confidence intervals that would result from observing varying numbers of symptomatic influenza events at a given dose level. The maximum CI width occurs at 50% observed events. With 30 subjects enrolled at the optimal dose, this would correspond to observing 15 symptomatic influenza events and an estimated CI of (31.3%, 68.7%).

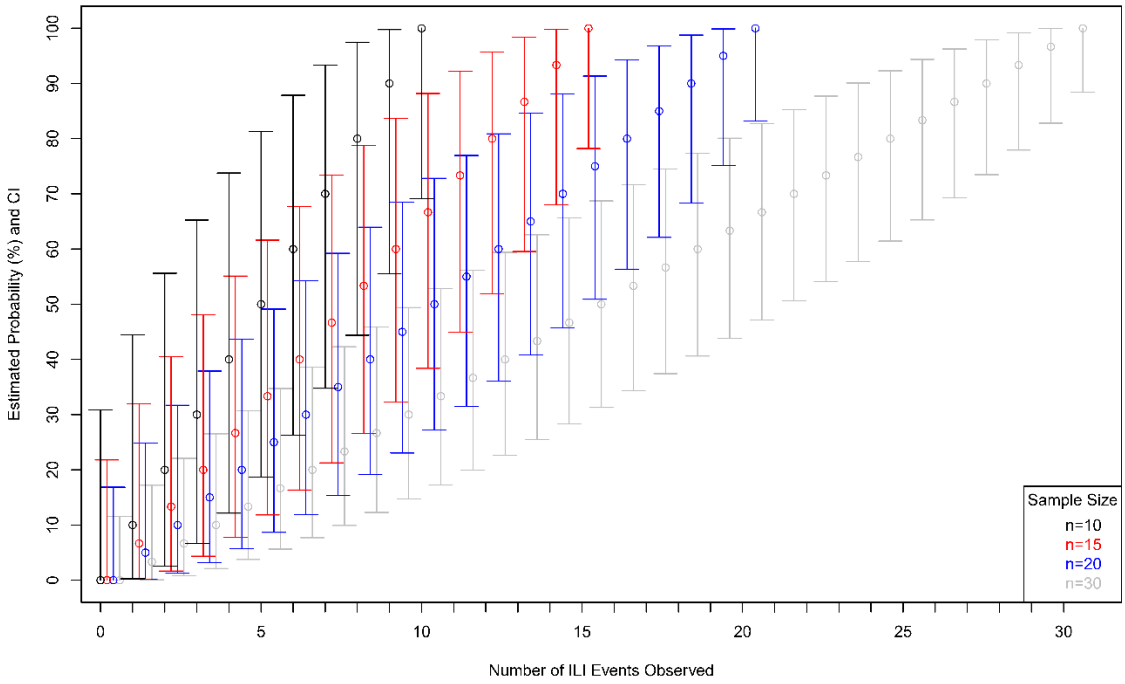

Figure 3. Estimated symptomatic influenza probabilities and associated exact 95% CIs, given varying numbers of symptomatic influenza events observed and varying sample sizes

Table 3 gives the probability of observing one or more safety events, such as a solicited symptom or an SAE of a particular classification in varying total dose group sizes, given underlying true event probabilities.

Table 3. Probability (%) of observing at least one safety event, given varying underlying event probabilities and dose group sizes

	Dose group Size			
True Event Probability	**N=10**	**N=15**	**N=20**	**N=30**
0.01 (Rare)	0.1	0.15	0.2	0.3
0.1 (Uncommon)	0.996	1.49	1.98	2.96
1 (Common)	9.56	14	18.2	26
10 (Very Common)	65.1	79.4	87.8	95.8

9.4 Populations for Analyses

- The Safety (SA) population will include all subjects who received study influenza challenge.
- The Intent-to-Treat (ITT) population will consist of all subjects who received study influenza challenge.
- The Per-Protocol (PP) population will include subjects in the ITT population with the following exclusions of data:
    - All data from any subjects who withdraw prior to the end of the challenge period
    - Data from any visit that occurs substantially out of window

- Data from all visits subsequent to major protocol deviations that could impact the validity of later data, such as:
  - 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

Certain analyses may be done on subsets of these populations, such as infected subjects, or those receiving what is determined to be the optimal dose.

9.5 Statistical Analyses

Clinical (symptom and infection) and safety data through the last follow-up at Day 57 will comprise the primary clinical database for this study. Once the last subject has completed the final visit, the primary clinical database will be cleaned, monitored, and locked. The clinical study report (CSR), comprised of the final analyses of safety, clinical, and available immunological data will be completed by the Statistical and Data Management Coordinating Center (SDMCC) after the primary clinical database is locked and the separate statistical analysis plan (SAP) has been 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 not available at the time of CSR preparation may be included in one or more addenda to the CSR.

Some analyses may be conducted on an expedited basis prior to the completion of the study and included in a "topline" report, to aid in decision-making regarding future studies. These analyses would not affect the conduct of this trial; subjects would be described by group but subject-level unblinding will not occur until the clinical database is locked. The topline results would be considered final and included in the CSR as well.

The formal SAP will elaborate on the analyses described here and will define which analyses would be included in an expedited report.

9.5.1 General Approach

For descriptive analyses, continuous variables will be summarized by the non-missing sample size, mean, standard deviation, and the minimum, median, and maximum. Categorical variables will be summarized by frequencies and percentages of observed levels, based on the non-missing sample size. Titers will be summarized with geometric means, first across replicates to compute one value for each individual sample and then applied again across subjects within the same group.

Generally, summaries will be presented by challenge dose group, with sham placebo subjects pooled across all challenge cohorts into one group. For safety endpoints, summaries will include a column for all subjects receiving active challenge virus as well. Other groupings used for some analyses may include symptomatic/asymptomatic, shedding virus yes/no (i.e., RT-PCR+/RT-PCR-), and symptomatic influenza+/symptomatic influenza- or MMID+/MMID-.

Data listings will be presented sorted by clinical site or other grouping variable, subject, and then by visit number within subject, where applicable. All tables will be annotated with the total

population size relevant to that table, including any missing observations. The analysis population for each exhibit will be clearly indicated.

While the study was not designed for any formal group comparisons, hypothesis testing will be done for some endpoints and will be considered exploratory. For hypothesis testing related to safety endpoints, two-sided tests at the α=0.05-level will be made without adjusting for multiple testing. For hypothesis testing of clinical endpoints, initially a global, two-sided test will be used to determine if there are differences between any groups. If the global test is significant at the α=0.05-level, the Bonferroni-Holm stepdown procedure [9] will be used to control the type-I error across pairwise two-sided tests, but no adjustment will be made across endpoints; a familywise 5% error rate will be used to determine significance. For immunological, genomic, or physiological analyses involving hypothesis testing, more stringent thresholds for significance may be used, depending on the number of hypothesis tests comprising a related set of analyses.

For hypothesis tests of continuous endpoints, ANOVA will be preferred for tests across multiple groups and t-tests for pairwise tests. If histograms of the analysis data do not appear approximately normal even after log- or other transformation and the sample sizes for comparisons groups are too small for the application of the central limit theorem (which states that in sufficiently large samples, the sample mean is approximately normal), Wilcoxon rank-sum tests or other nonparametric methods may be utilized instead. For tests of categorical endpoints, exact binomial tests or Fisher's exact tests will be used.

Exact Clopper-Pearson confidence intervals will be computed for binary variables, where applicable, and for continuous variables confidence intervals will be computed based on the t-distribution with n-1 degrees of freedom, where n denotes the number of observations.

If other analysis approaches than those listed here are used, they will be clearly stated and justified in the SAP and/or CSR.

9.5.2 Analysis of the Primary Endpoints

The three primary objectives for this study involve characterizing the infectivity and the symptom profile elicited by increasing doses of the study challenge virus, as well as determining the optimal dose for use in future challenge and vaccine studies.

The primary endpoints are:

- The percentage of subjects within a challenge dose group with detectable shedding by qualitative RT-PCR and symptom scores that meet the clinical case definition for symptomatic infection by the Modified Jackson Instrument score
- The percentage of subjects within each challenge dose group with detected viral shedding, the magnitude of viral shedding from Day 2 through Day 8, and the duration of viral shedding
- The computed symptom score from the modified Jackson symptom score, and the percentage of subjects with total symptom scores meeting criteria for symptomatic or asymptomatic categories

The probability of symptomatic influenza will be estimated for each challenge dose group, along with associated 95% exact confidence intervals. The optimal infectious dose will be the challenge dose group with the greatest estimated probability of symptomatic influenza which is

safe, as determined by the study halting criteria. If doses with similarly high observed AR have substantially different group sizes (e.g., 10 vs. 30), the lower limit of the estimated 95% CIs for the AR may be considered as well.

Viral shedding will be summarized by the number and percentage of subjects in each challenge dose group with positive qualitative and/or quantitative RT-PCR each day from Day 2 through the end of the challenge period (Day 8 or later, in the case of a subject not meeting discharge criteria on Day 8) excluding safety results reported after receipt of antiviral therapy. The probability of viral shedding at any point during the challenge (i.e., incidence) will be estimated along with the associated exact 95% CI, separately by challenge dose group. Peak viral shedding will be calculated for each subject as the maximum shedding from Day 2 through Day 8 or discharge, and total viral shedding will be calculated via the area under the curve (AUC) with study day on the x-axis and shedding on the y-axis. These will be calculated separately for quantitative RT-PCR and quantitative culture. Mean peak and total shedding based on the AUC will be calculated for each group along with 95% CIs, and comparisons will be made as described for continuous variables in Section 9.5.1. A Wilcoxon signed-rank test will be used to compare distributions of these variables between quantitative RT-PCR and quantitative culture across all subjects, to assess if these methods of viral quantification are consistent. Scatterplots will also be presented, along with Spearman rank-order correlation estimates. Duration of viral shedding will be calculated for each subject as the number of days (including non-consecutive) determined by qualitative or quantitative RT-PCR to be positive from Day 2 until discharge; means and 95% CIs will be calculated for each group and comparisons will be made.

Solicited influenza symptoms will be presented by the frequency and percentage of subjects reporting each symptom, severity, day, and challenge dose group. The self-reported (FLU-PRO) and investigator-assessed symptoms will be tabulated separately. Total symptom scores will be computed for each subject and each scoring algorithm. Mean scores for both algorithms will be calculated for each group, along with 95% CI, and the groups will be compared as described in Section 9.5.1. Additionally, the number and percentage of subjects meeting each scale's definition of symptomatic and asymptomatic will be cross-tabulated by challenge dose group. A Wilcoxon rank-sum test will be used to compare the distributions of each set of scores, across all subjects that received active challenge, to determine the agreement between the two symptom reporting methods.

Clinical endpoints (including the primary endpoints) will be assessed in the ITT population. Subjects who withdraw during the challenge period prior to meeting discharge criteria will be included in summaries where data are available. Subjects who withdraw during the challenge period and have yet to meet the relevant clinical case definition will be excluded from analyses of aggregate endpoints such as the incidence of symptomatic influenza.

If at least 10% of the analysis data would be dropped according to PP population exclusions described in Section 9.4, the PP population will be used as a sensitivity analysis for the primary ITT analysis.

9.5.3 Analysis of the Secondary Endpoints

9.5.3.1 Analysis of Secondary Endpoints

Assessing the safety profile of the challenge virus is a secondary objective of the study. This will be measured by the number of adverse events reported and the percentage of subjects reporting adverse events through Day 29, and the number of serious adverse events and the percentage of subjects reporting adverse events through Day 57.

Influenza signs and symptoms and clinical lab findings determined to have exceeded the expected severity will be considered unsolicited adverse events. Events that are not consistent with illness due to influenza and abnormal vital signs will be considered unsolicited AEs as well. Solicited signs and symptoms of influenza as described in Section 4.1 will not be considered AEs. The severity (mild, moderate, or severe) of abnormal vital signs and clinical laboratory values will be presented according to the toxicity grading scales in Appendices A, B and C. Other events will be reported according to the criteria in Section 8.3.2.1. Relationship with receipt of the challenge virus will be assessed as described in Section 8.3.2.2.

The frequencies of AEs through Day 29 and SAEs through Day 57 will be presented by MedDRA® system organ class (SOC) and preferred term (PT), severity, relationship to challenge virus, challenge dose group, and across all subjects receiving active virus. Incidence will be assessed by the number and percentage of subjects in each challenge dose group ever reporting an event, along with associated 95% CI for the probability of the event. To further assess the safety of the challenge virus, the incidence of any related moderate or severe AE and any SAE will be calculated for each challenge dose group and compared as described in Section 9.5.1.

Adverse events and clinical laboratory results will be presented in data listings. Analyses of safety endpoints will be performed for the Safety population.

9.5.3.2 Analysis of Secondary Immunological Endpoints

Secondary immunological objectives include describing the host serum antibody (HAI and MN) responses and anti-HA-stalk antibody responses at baseline and post-challenge.

For these titer data, GMTs and the proportions of subjects achieving seroconversion, defined as a minimum 4-fold rise in titer post-challenge, will be presented by challenge dose group and visit. Geometric mean fold-rise (GMFR) for anti-HA-stalk antibody titers will be included for post-challenge visits as well. Confidence intervals corresponding to these statistics will be computed for each group and visit as well. Hypothesis testing may be performed for select immunological endpoints and time points, which will be pre-specified in the SAP.

9.5.4 Analysis of Exploratory Endpoints

Exploratory objectives for this study include further characterizing the immunological responses, assessing the impact of genetic variability on the immune, clinical, and viral responses, and further examining the clinical and physiological responses post-challenge.

9.5.4.1 Exploratory Immunological Endpoints

For analysis of exploratory immunological endpoints, immune responses will be described by visit and challenge dose group. For antibody titer data, geometric means will be computed for each group at each time point (GMTs), and seroconversion and geometric mean fold-rise (GMFR) at post-baseline visits will be calculated as well, where applicable. Confidence intervals corresponding to these statistics will be computed for each group as well, but formal hypothesis testing is not planned for descriptive summaries of exploratory endpoints.

Additional aggregate (i.e., encompassing data across multiple visits) immunological endpoints may include the duration of response, the timing and magnitude of the peak response, and the total immune response as measured by the area under the curve (where the study day is on the x-axis and the immune response is on the y-axis).

Neuraminidase inhibition antibody (NAI) responses will be analyzed similarly to HAI and MN assays, as described in 9.5.3.2. Other immunological endpoints will include frequencies of cell subsets, which will be described by mean frequencies within each group and the associated 95% CIs. Further details for data types not covered here will be given in the standalone SAP.

Exploratory immunological endpoints will be analyzed for the ITT population, with the PP population used for sensitivity analysis if at least 10% of analysis data would be excluded for the reasons described in Section 9.4. Subjects who withdraw prior to being evaluable for certain endpoints may be excluded from specific analyses in the ITT population, but all available data will be included in descriptive analyses.

9.5.4.2 Exploratory Genomic Endpoint

The frequencies of HLA class I and II alleles at baseline will be tabulated by challenge dose group and overall. The ability of pre-challenge HLA class I and II alleles to modulate the magnitude (as measured by serological conversion defined as a minimum 4-fold rise in post-challenge) of the immune response will be assessed using a two-sided Fisher's exact test. The relationship between HLA alleles and pre-existing immune status (baseline HAI, NAI, MN titer) will be evaluated via one-way ANOVA. The association between HLA class I and II alleles and peak shedding and total viral shedding for quantitative RT-PCR and quantitative culture will be assessed using a two-sided Wilcoxon Rank-Sum test. Depending on the number of observed alleles, multiple testing adjustment may be carried out using the Benjamini Hochberg procedure[10].

9.5.4.3 Exploratory Transcriptomic Endpoint

For the transcriptomics exploratory analysis, RNA 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*. Gene expression quantification will be carried out by using the *Subread* software using the latest Ensembl [11] gene model annotations as a reference. Systematic differences in sequence coverage between samples will be accounted for using the TMM method [12] as implemented in the *edgeR* [13] 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* [13] 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[10] 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* [14] 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 [15] and KEGG [16] databases as well as Blood Transcription Modules [17] accounting for gene length bias will be carried out using the implementation provided by the *goseq* [18] 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.

9.5.4.4 Exploratory Physiological and Additional Clinical Endpoints

Additional exploratory analysis of clinical endpoints will include cross-tabulations of symptoms as collected by each of the two scoring systems, separately by viral shedding status (as determined by RT-PCR), by receipt of active challenge virus or placebo, and across all subjects. To examine the ability of each scoring algorithm to accurately capture symptomatic influenza/MMID, the symptom scores (i.e., severity) for each symptom will be summarized by RT-PCR status. These summaries will be made on a by-visit level and for the maximum severity/score of each symptom over the entire challenge period.

Alternative case definitions for symptomatic influenza virus infection will be explored via development of clinical scoring algorithms based on each of the scoring scales. A training and testing approach will be utilized to determine multi-symptom models predictive of RT-PCR positivity, using the maximum severity observed over the challenge period for each symptom. This approach will be applied separately for each scoring scale, and the operating characteristics of the final multi-symptom models will be compared.

Physiological data, as collected by the wearable devices, will be summarized by dose group and by symptomatic influenza status, separately. Further exploratory analysis of these data will be described in the SAP.

9.5.5 Baseline Descriptive Statistics

Baseline and demographic characteristics will be summarized by clinical site for each challenge dose group as well as the placebo group with summary statistics as described in Section 9.5.1. These characteristics will include age, sex, ethnicity, race, and prior receipt of seasonal vaccination.

9.5.6 Planned Interim and Early Analyses

After each cohort has finished the challenge period of the study, the symptomatic influenza AR will be analyzed and the decision to keep the same dose or escalate to the next dose for the

subsequent cohort will be made according to the criteria given in **Figure 1**. A safety monitoring committee (SMC) will be convened, and the SMC will meet and review these data at scheduled time points or ad hoc as needed during this study, as delineated in the SMC charter.

A set of analyses may be included in an expedited report prior to final database lock, as described in Section 9.5. Care would be taken to ensure that dissemination of results prior to the end of follow-up would not impact study conduct.

9.5.6.1 Interim Safety Analyses

An interim safety review may include enrollment and demographic information, clinical laboratory tests, and AE/SAEs. Additional data may be requested by the SMC. The review(s) will be blinded to active virus or placebo, with unblinded study assignments available upon SMC request.

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 7.1.1 are met, and if so, the SMC will be convened to review.

9.5.6.2 Interim Review to Determine the Dose for the Subsequent Cohort

After all subjects from each challenge cohort have been discharged from the challenge unit, the infection, symptom, and safety data will be cleaned to prepare for the interim review to determine the dose for the next challenge cohort enrolled.

If the minimum symptomatic influenza AR threshold given in **Figure 1** is met and no halting criteria for safety have been met, the next group of subjects will be enrolled at the same dose level. If the threshold is not met and no halting criteria have been met, the next cohort will be enrolled at the next highest dose level. If the halting criteria are met, the SMC will review to determine whether the study should be halted due to safety concerns.

Data will be considered in aggregate as part of the interim reviews for dose selection, in order to minimize the potential for unblinding.

9.5.6.3 Early Analyses

As described in Section 9.5, some analyses may be completed prior to the end of follow-up and/or the clinical database lock, to facilitate planning for future studies. The analyses intended for inclusion in an expedited topline report will be delineated in the SAP, along with operational details for maintaining the blind and the integrity of the rest of the study.

9.5.7 Sub-Group Analyses

No explicit sub-group analyses beyond those relating to the dose of study challenge received are planned. However, exploratory analysis may be conducted to examine potential impacts of baseline characteristics such as sex or baseline HAI, NAI, or MN titers.

9.5.8 Tabulation of Individual Participant Data

Safety data, clinical data, and immunological data will be presented in listings, in addition to the planned analyses by dose group.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

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. and provide the FWA number to DMID.

A single IRB will conduct the ethical review and approval for participating sites. 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.

10.1.1 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation.

Pre-screening may occur under an active screening protocol (DMID 20-0004). Informed consent for the current protocol will be obtained at the time of screening Days -45 to -3 and documented prior to performing any study procedures. 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. Additional screening activities 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.

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 for future use of specimens for secondary research. Please refer to Section 10.1.4. The subject will be asked to consent specifically to genetic testing (HLA testing) as well as 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 trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. 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 trial 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 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.

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 trial 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 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 clinical trial 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 allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial 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 trial. A copy of the signed consent form will be given to the subject(s) for their records.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated, and subjects will be re-consented per IRB requirements, if necessary.

10.1.1.1 Other Informed Consent Procedures

The rights and privacy of human subjects who participate in genomic or phenotypic research studies will be protected at all times. The consent process, including relevant language in the ICF will provide an explanation of the potential risks to the individual study subjects and their families. The consent will include whether individual subject will be shared through a NIAID-designated controlled access data repository. Clinical metadata, genomic, or other datasets or a subset of the clinical and other metadata that may potentially identify human subjects will not be released in unrestricted databases. Subjects will be informed that the evolution of genomic technology and analytical methods raises the risk of re-identification, even when specimens are de-identified. The subject will be asked to consent for future use of specimens for secondary research; genetic testing on these samples may be performed specifically to genetic testing (HLA testing and transcriptomics) planned for this study. Please refer to Section 10.1.4.1.

In addition to this protocol, subjects will be asked to consent to a separate biorepository protocol for the use of residual samples and associated data in future research.

10.1.2 Study Termination and Closure

Section 7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal, describes the temporary halting of the study.

This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Halting criteria met and it is deemed unsafe to proceed with further dosing with influenza challenge
- Insufficient compliance with protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Decision by regulatory authorities

If the study is prematurely terminated, the Principal Investigator (PI) will promptly inform study participants and the Institutional Review Board (IRB) and regulatory authorities as applicable. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. The PI will assure appropriate follow-up for the subjects, as necessary.

The sponsor will notify regulatory authorities as applicable.

10.1.3 Confidentiality and Privacy

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to participants, test results of biological samples and genetic tests, and all other information generated during participation in the study. No identifiable information concerning participants in the study will be released to any unauthorized third party. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or 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 participants in this study. The clinical study site will permit access to such records.

All source records including electronic data will be stored in secured systems in accordance with institutional polices and federal regulations.

All study data and research specimens that leave the site (including electronic transmission of data) will be identified by a coded number that is linked to a subject through a code key maintained at the clinical site. The code key, including names or readily identifiable information is kept confidential and will not be transmitted off site.

As this research is funded by the NIH, it is covered by NIH policy which effectively issues the research a Certificate of Confidentiality. By this policy, researchers cannot be forced to disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains.

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 participant 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 the release is in compliance with applicable Federal regulations governing the protection of human subjects in research.

10.1.4 Future Use of Stored Specimens and Data

Secondary Human Subject Research is the re-use of identifiable data or identifiable biospecimens that were collected from some other "primary" or "initial" activity, such as the data and samples collected in this protocol. This section will detail the samples and data available for secondary research. Any use of the sample or data, however, will be presented in a separate protocol and require separate IRB approval.

10.1.4.1 Samples for Secondary Research

Samples will not be collected for planned secondary research. However, leftover research samples after the laboratory testing specified in this protocol is completed will be stored for potential future studies with the subject's consent. Specimens will be coded. Any future testing laboratory will not have access to the code, and therefore will not be able to identify study participants. Genetic testing on these samples may be performed. The specifics of this testing will be in a separate protocol and will be reviewed by an IRB.

The following types of samples will be stored and used for secondary research:

- Residual biological specimens- Any leftover primary research sample after laboratory testing is completed per protocol and which the protocol explicitly states are allowed to be stored and used for secondary research, and for which the research subject gave specific consent.
  Repository research sample- Samples will be collected with the subject's consent in this protocol with the intent to store for additional research (i.e., samples collected beyond those needed for primary research) and will be used in future studies.

In addition to this protocol, subjects will be asked to consent to a separate biorepository protocol for the use of residual samples and associated data in future research.

To participate in this study, subjects will be asked for consent for storage of samples for secondary use. Samples will be stored indefinitely at a DMID-designated storage facility. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. Secondary research with coded samples and data may occur, however, subject confidentiality will be maintained as described for this protocol. An IRB review of the secondary research using coded specimens is required.

Residual/Repository Research Samples, upon written request and approval from DMID and any approvals required by the site, may be shared for secondary research with investigators at the participating site, with researchers at other CIVICs sites or other institutions, or company-designated research laboratories. The samples will not be sold or used directly for production of any commercial product. DMID will authorize shipment from the repository.

Reports from secondary research will not be kept in the subjects' health records or shared with subjects, unless required by law. Reports will not be sent to the specimen repository.

The subject's decision for secondary research can be changed at any time by notifying the study doctors or nurses in writing. If the subject changes his/her decision, the samples will be destroyed if the samples have not been used for research or released for a specific research project.

10.1.4.2 Data Sharing for Secondary Research

Data from this study may be used for secondary research. All of the individual participant data collected during the trial will be identified by a coded number that is linked to a subject through a code key maintained at the clinical site. The code key is kept confidential and will not be transmitted off site. All of the individual participant data collected during the trial will be made

available after deidentification. The Protocol, Statistical Analysis Plan, and Analytic Code will also be made available. This data will be available immediately following publication, with no end date.

The investigator may request removal of data on individual study subjects from NIH data repositories in the event that a research subject withdraws or changes his or her consent. However, some data that have been distributed for approved research use cannot be retrieved.

10.1.5 Key Roles and Study Governance

The study is sponsored by DMID. Decisions related to the study will be made by the protocol team, which includes representatives from the participating sites (PI), DMID (sponsor), and the SDMCC. Key study team members and roles are listed in the Manual of Procedures (MOP).

10.1.6 Safety Oversight

This clinical study will utilize an internal safety review committee (ISRC), consisting of the medical monitor, medical officer, the PIs from both sites and a representative from the SDMCC. The ISRC will meet following Day 8 for each dosing cohort to review safety events and assure no halting criteria have been met and to assess the attack rate for each cohort to determine how to proceed with either remaining at the same challenge dose or escalating to the next highest dose. If one of the halting criteria has been met, the SMC will be convened.

As noted above, 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
- A final meeting to review cumulative unblinded safety data in a standard summary format.

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review applicable data including, but not limited to, enrollment, demographics, dosing, data, laboratory data and safety data at scheduled time points during this trial as defined in the SMC charter. The

SMC will review blinded aggregate data in the open session of the SMC meetings. Unblinded data (grouped by treatment) will be reviewed in the closed session only.

Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study drug administration, and to continue, modify, or terminate this trial.

10.1.7 Clinical Monitoring

Site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently IRB/IEC approved protocol/amendment(s), ICH GCP, and applicable regulatory requirement(s). Clinical monitoring also verifies any critical study procedures are completed following specific instructions in ancillary documents referenced in the protocol such as a Manual of Procedures.

Monitoring for this study will be performed by DMID. Details of clinical site monitoring are documented in a CMP. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study staff and all study documentation according to the DMID-approved CMP. Study monitors will meet with all participating site PIs to discuss any problems and outstanding issues and will document site visit findings and discussions.

10.1.8 Quality Assurance and Quality Control

To ensure the reliability of study data, each site will develop a Clinical Quality Management Plan (CQMP). The CQMP will describe

- Routine internal quality control (QC) and quality assurance (QA) activities
    - For the purposes of measuring, documenting and reporting study conduct, protocol adherence, human subjects' protections, and reliability of the protocol-driven data collected.
- A process for addressing in a timely manner any data quality issues (i.e., collecting, recording and reporting data), systemic issues (i.e., protocol conduct, non-compliance, human subject protections), and implementation of CAPA procedures.

The SDMCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the SDMCC to the sites to record and maintain data for each subject enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Clinical research data (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments), and clinical laboratory data will be abstracted from the source documentation and/or collected on the data collection form 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.

Data reported in the eCRF should be consistent with the data collection form/source documents or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

All data collection forms, and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Company, Inc., under subcontract to Digital Infuzion, Inc. will serve as the Statistical and Data Management Coordinating Center for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

10.1.9.2 Study Record Retention

Study related records, including the regulatory file, study product accountability records, consent forms, subject source documents and electronic records should be maintained for a period of 2 years following the date a marketing application is approved for the investigational 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 such indication, until 2 years after the investigation is discontinued and FDA is notified. These documents should be retained for a longer period, however, if required by local policies or regulations. No records will be destroyed without the written consent of DMID. Consent forms with specimen retention linked to identifiable

specimens will be maintained for as long as the specimens remain in identifiable format, and a minimum of three years after use of the identifiable specimens in nonexempt human subject research. Both sites must contact DMID for authorization prior to the destruction of any study records.

10.1.9.3 Source Records

Source data are all information in original records (and certified copies of original records) of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP, regulatory, and institutional requirements. For this trial, hardcopies of the study visit worksheets will be provided for use as source document worksheets. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, any process that is noted in the protocol and refers to details in the protocol-specific MOP, or GCP requirements or any critical study procedures with specific instructions in ancillary documents referenced in the protocol such as a protocol-specific MOP.

The noncompliance may be either on the part of the subject, the investigator, or the study site staff. Following a deviation(s), corrective actions should be developed by the site and implemented promptly. All individual protocol deviations will be addressed in subject study records.

It is the responsibility of the site principal investigator 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 via Advantage eClinical (IDES). Protocol deviations must be sent to the IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements. All deviations from the protocol must be addressed in study 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 if the deviation is subject specific.

10.1.11 Publication and Data Sharing Policy

Following completion of the study, results of this research will be published in a scientific journal.

Data will be available immediately following publication, with no end date, with data sharing at the discretion of the Sponsor. Sites may also obtain individual or country level data from the database for separate publications is desired. Publication may occur prior to completion of a final clinical study report for the entire trial.

10.1.12 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.13 Genomic Data Sharing Policy

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human data, as well as the use of these data for subsequent research.

10.1.14 Publications

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. This study will adhere to the following publication and data sharing policies and regulations:

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. As such, the final peer-reviewed journal manuscripts will be accessible to the public on PubMed Central no later than 12 months after publication.

10.1.15 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. DMID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Additional Considerations

10.2.1 Research Related Injuries

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 by the NIAID, NIH, or the federal government to the subject, for any injury suffered due to participation in this trial.

10.3 Abbreviations

Table 4. Commonly Used Abbreviations

ADL	Activities of Daily Living
AE	Adverse Event
ALC	Absolute Lymphocyte Count
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of Covariance
AR	Attack Rate
BLA	Biologics License Applications
BMI	Body Mass Index
CAPA	Corrective and Preventative Action Plan
CFR	Code of Federal Regulations
CHI	Controlled Human Infection
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CMS	Clinical Material Services
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
Cr	Creatinine
CRO	Contract Research Organization
CSR	Clinical Study Report
CXR	Chest x-ray
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
g/dL	Grams per Deciliter
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HA	Hemagglutination
HAI	Hemagglutination Inhibition Test
HBV	Hepatitis B

HCV	Hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
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
ISO	International Organization for Standardization
ISRC	Internal Safety Review Committee
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MMID	Mild-to-Moderate Influenza Disease
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
N	Number (typically refers to subjects)
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NLF	Nasal Lavage Fluid
NSAID	Non-Steroidal Anti-inflammatory Drug
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PA	Posterior anterior (refers to CXR view)
PE	Physical Exam
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PPG	Photoplethysmography
QA	Quality Assurance
QC	Quality Control
RG	Reverse genetics
RR	Respiratory Rate
RT-PCR	Reverse Transcription – Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDMCC	Statistical and Data Management Coordinating Center
SE	Standard Error

SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SPG	Sucrose phosphate glutamate
SpO_2	Saturation of Peripheral Oxygen
$TCID_{50}$	Median Issue Culture Infectious Dose
ULN	Upper Limit of Normal
UP	Unanticipated Problem
US	United States
VTEU	Vaccine and Treatment Evaluation Unit
WBC	White Blood Cell

10.4 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
2.0	19Jul2021	Subjects will be pre-screened for study inclusion to have serological HAI antibody titers of ≤1:40 against the clinical challenge strain instead of ≤1:10.	To expand enrollment efforts
		Potentially eligible study subjects may be pre-screened under an active screening protocol (DMID 20-0004) instead of will.	Administrative
		The MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, Morrisville NC) will be sourced by the sites instead of by DMID Clinical Material Services (CMS).	Administrative
		Deleted "The MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, Morrisville NC) will be stored at room temperature (15-30°C)." as this sentence was repeated twice in the same Section 6.2.3.	Administrative
		Detailed instructions for challenge virus inoculum preparation are provided in the MOP.	Administrative
		Each challenge cohort will be enrolled at one of the clinical sites instead of in an alternating fashion.	Administrative
3.0	20Sep2021	Clarified eligibility criteria	Administrative
		Added option for licensed study clinicians listed on the Form FDA 1572 to conduct various study procedures	Administrative
		Removed information regarding enrollment at one of the clinical sites	Administrative
		Clarified approximate cohort sizes	Administrative

		Updated wearable device language to allow for another device	Administrative
4.0	29Oct2021	Updated venipuncture volumes and SoA to collect blood on Days 5 and 7	Procedural
		Removed additional multiplex requirement at 72 hours prior to confinement	Procedural
		Modified exclusion criterion to exclude individuals that participated in flu challenge study with flu virus of same subtype within past 2 years	Administrative
		Removed Modified Jackson score from appendices. Reference in Emmes DCFs	Administrative
		Added language to allow for initiation of antiviral treatment course on Study Day 6	Procedural
5.0	26Jan2022	Updated exclusion criterion #9 to relax requirement from 6 months to 4 months for prior receipt of flu vaccination.	Administrative
		Added UMB's proposed mass cytometry work	Procedural
		Clarified IP temperature storage language	Administrative
		Clarified grading for abnormal lab ranges so that ULN and LLN will not be considered abnormal	Administrative
6.0	17Feb2022	Modified study product preparation sections to refer to the MOP for details.	Procedural
		Updated venipuncture volumes for EDTA tubes.	Administrative
		Corrected spelling, grammar, and formatting errors.	Administrative
7.0	11Apr2022	Updated inclusion criterion #8 and #9 to allow for select drug use	Administrative
		Updated language regarding confinement of backup subjects	Administrative
8.0	02May2023	Updated objectives and endpoints to move NAI assay from	Administrative

		secondary to exploratory objectives and associated changes in text in Section 9.	

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12. APPENDIX A. SEVERITY GRADING FOR VITAL SIGNS

- Oral temperature will be graded as follows:

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, and Respiratory Rate will be graded as follows:

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
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

13. APPENDIX B. SEVERITY GRADING FOR SAFETY LABORATORIES

- Clinical safety laboratory values will be graded as follows:

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
WBC x10^3/µL (Decrease)	2.50 – <LLN	1.50 – 2.49	<1.50
WBC x10^3/µL (Increase)	>ULN – 15.09	15.10 – 20.00	>20.00
Absolute Lymphocyte count x 10^3/µL (Decrease)	0.50 – <LLN	0.40 - 0.49	<0.40
Hgb g/dL (Decrease) (Female)	10.1 – <LLN	8.5 – 10.0	<8.5
Hgb g/dL (Decrease) (Male)	11.0 – <LLN	9.5 – 10.9	<9.5
Platelets cell x 10^3/µL (Decrease) EDTA	120 – <LLN	100 – 119	<100
Platelets x 10^3/µL (Increase) EDTA	>ULN – 550	551– 750	>750
Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
ALT IU/L (Increase) (Female)	>ULN – 100	101 – 200	>200
ALT IU/L (Increase) (Male)	>ULN – 138	139 – 275	>275
Creatinine mg/dL (Increase)	>ULN – 1.8	1.9 – 2.2	>2.2

14. APPENDIX C: VENIPUNCTURE VOLUMES

	Study Screening	**Pre-Challenge**		**Viral Challenge**	**Post- Challenge**										**Cumulative Blood Volume Total (mL)**	*Unplanned visits*[10]		
		Confinement period										**Follow-up**				*Extended Confinement*[11]	*Early termination*	*Unscheduled*
Study Visit Number	**00A**	**00B**	**00C**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**				
Study Day	**D -45 to -3**	**D -2**	**D -1**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D15 ± 3**	**D29 ± 5**	**D57 ± 7**		*D9+*		
Blood for Safety Lab Evaluations (Screen)[1]	18.5														**18.5**			
Blood for Safety Lab Evaluations (Follow-up)[2]					8.5		8.5				8.5				**25.5**	*8.5*	*8.5*	
HAI Serology Screen	8.5														**8.5**			
Serological Assays[3]			20								20	20	20	20[9]	**100**	*10*	*20*	*20*
Blood for plasma and PBMC for T- and B-cell immunology[4,5,6]			24								24	24	24	24	**120**		*24*	*24*
Blood – PBMC for plasmablasts[7]											24				**24**		*24*	
Blood – PBMC for immunophenotyping[8]			24		24		24		24		24		24		**144**		*24*	*24*
Serum for cytokine assays		5	5		5	5	5	5	5	5	5	5			**50**	*5*	*5*	
Blood – RNA transcriptomics		5	5		5	5	5	5	5	5	5	5			**50**	*5*	*5*	
Blood – DNA for genetic testing			4												**4**			

	Study Screening	Pre-Challenge		Viral Challenge	Post- Challenge										Cumulative Blood Volume Total (mL)	*Unplanned visits*[10]		
		Confinement period										Follow-up				*Extended Confinement*[11]	*Early termination*	*Unscheduled*
Study Visit Number	**00A**	**00B**	**00C**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**				
Study Day	**D -45 to -3**	**D -2**	**D -1**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D15 ± 3**	**D29 ± 5**	**D57 ± 7**		*D9+*		
Per Visit Blood Volume Total (mL)	**27**	**10**	**82**	**0**	**42.5**	**10**	**42.5**	**10**	**34**	**10**	**110.5**	**54**	**68**	**44**	**544.5**	**28.5**	**110.5**	**68**

[1] HIV, Hepatitis B surface antigen, Hepatitis C antibody, White blood cells (WBCs), absolute lymphocyte count (ALC), hemoglobin (Hb), platelets (PLT), alanine transaminase (ALT), creatinine (Cr), serum HCG (in females only).
[2] White blood cells (WBCs), absolute lymphocyte count (ALC), hemoglobin (Hb), platelets (PLT), alanine transaminase (ALT), creatinine (Cr)
[3] HAI = hemagglutination inhibition; NAI = neuraminidase inhibition; MN = microneutralization; or anti-HA-stalk
[4] Frequency of influenza-specific B cell subsets in circulation
[5] Magnitude of influenza-specific T cell responses in circulation (PBMC) to conserved T cell epitopes and inactivated virus
[6] Presence of hemagglutinin (HA)-specific CD4 T cells with a focus on Tfh cells. This will be done on a subset of subjects.
[7] A/Texas/71/2017/H3N2-specific plasmablasts, their specific-antibody (e.g., anti-H3, -N2 and – H3N2 virus) production and isotype (e.g., IgG, IgA)
[8] The frequency, duration, timing and phenotypic characteristics of immune cells in the blood
[9] To be collected at this time point or latest available time point
[10] These collections will only be taken in place of those from planned visits. The total volume will not be larger than the cumulative blood volume total shown.
[11] These collections will only occur if a subject remains in confinement past Study Day 8 and if clinically indicated.

15. APPENDIX D: FLU-PRO – PARTICIPANT REPORTED TOOL

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)	☐	☐	☐	☐	☐

Stomachache	☐	☐	☐	☐	☐

Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐

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?	☐	☐	☐	☐	☐

Participant ID: _____ **Participant Initials:**_____ **Date:**_____/_____/_____

Items to be asked in the daily diary through to Day 15 along with the Flu-PRO items.

1. 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

2. [Skip this question if you answered 0 above]. 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

3. [Skip this question if you answered 0 in Question #1]. How much did your flu symptoms interfere with your activities today? (Please select one response only)
- 1 Not at all
- 2 A little bit
- 3 Somewhat
- 4 Quite a bit
- 5 Very much

4. Have you returned to activities today?
- 0 No
- 1 Yes

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

6. Are you in your usual state of health today?
- 0 No
- 1 Yes