CLINICAL STUDY PROTOCOL

Phase 1b clinical study to investigate the safety and immunogenicity of the H3N2 Sing2016 (A/Singapore/INFIMH-16-0019/2016) M2SR monovalent influenza vaccine in adults ages 50 to 85 years old

Product	Sing2016 M2SR Vaccine
Protocol Number	FLUGEN-H3N2-V005
IND Number	016968
Clinical Phase	1b
Clinical Indication	Not applicable (Healthy Volunteers)

Initial Protocol Version 3.0, 9-Sep-2021

CONFIDENTIALITY STATEMENT

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This study will be conducted in compliance with this protocol, the ICH Note for Guidance on Good Clinical Practice (CMPM/ICH/135/95) and with the applicable regulatory requirement(s)

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CLINICAL STUDY PROTOCOL AMENDMENT

SUMMARY OF CHANGES AND RATIONALE

FLUGEN-H3N2-V005 Amendment 3

9 September 2021

Protocol FLUGEN-H3N2-V005 Amendment 2 (dated 10 May2021) has been modified by Amendment 3 to incorporate the changes listed below. Minor edits and typographical corrections have also been made as needed.

1. **Exclusion Criteria:** (Sections revised: Section 4.2 – Subject Exclusion Criteria)
   Individuals with Grade 2 elevated cholesterol may be enrolled at the discretion of the Investigator, if their overall lipid panel does not indicate an increased risk for cardiovascular disease.

   Rationale: Language removed from the protocol since lipid panel is not collected in the study.

2. **Exclusion Criteria**: (Sections revised: Section 4.2 – Subject Exclusion Criteria)
   Donation of blood or blood product of at least approximately 1 pint (~470mL) within 60 days of IP receipt or planned within 3 months (84 days) after IP administration.

   Rationale: Minor edit to the section.

3. **Vial Weighing**: (Sections revised: Section 5.1 – Study Vaccine, Section 5.7.1 – Investigational Vaccine)

   Devices will be weighed pre and post fill to allow calculation of dose filled into devices.

   Rationale: Language removed from the protocol as devices will not be weighed.

4. **Prior and Concomitant Therapy**: (Section 5.9 Prior and Concomitant Therapy)
   Subjects may receive a vaccine that is licensed or under an EUA if it is the opinion of the investigator that such treatment is appropriate and necessary. The preference, when possible, is to avoid such treatment during the 28 days post-visit 01.

   Rationale: Added language to provide clarification regarding vaccine administration during the study participation.

5. **Schedule of Events**: (Section revised : Section 6.1.1 Table 2: - Schedule of Events – IP Administration)

   Updated Schedule of events

   Rationale: Update to the schedule of events table to remove V01 hematology and chemistry (Footnote remains to provide additional clarification). Removal of vial weights.

6. **Screening Period**: (Section revised: Section 6.1.2 Screening Period)

Subjects will be re-confirmed for eligibility on Day 1 (V01) prior to dose administration. Chemistry and hematology at V00 will serve as eligibility and baseline values unless collected more than 28 days prior to V01. In such case the lab tests will be redone to establish baseline only.

Rationale: Added language to provide clarification regarding safety lab collection.

7. **Urine Samples**: (Section revised: Section 6.2.1.6 Urine Samples)

   Results are obtained by comparing the color block on the canister to those on the reagent strips where the color blocks enclosed by the black box are considered Normal. If dipstick urinalysis is Abnormal/positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination for white blood cells (WBC), red blood cells (RBC), bacteria, casts, and epithelial cells will be performed. Crystals, casts, and bacteria will only be reported if present.

   Rationale: Added language to provide clarification regarding urine sample collection.

8. **Bioanalysis**: (Section revised: Section 6.2.2 Bioanalysis)

   Blood samples for determination of the concentration of analytes specified in Appendix 3 will be analyzed by a qualified vendor under the responsibility of the Sponsor, using validated analytical methods.

   Rationale: Language previously stated "Blood and urine samples…" however urinalysis is being performed by use of dipstick.

9. **IP Doses and Vial Weights**: (Section revised: Section 6.2.6 IP Doses and Vial Weights) *The weight of each M2SR vaccine vial will be collected prior to and after filling of delivery devices. The weights will be recorded at the time point specified in the Schedules of Events – IP Administration (Table 2). A calculation of the original vial weight minus the emptied vial weight will indicate the weight of dose that was filled into the device and will be used to determine volume dispensed. The unblinded pharmacist will maintain the weight logs in a secure and confidential manner until after database lock to maintain the blind. The weight logs will be periodically reviewed by the unblinded pharmacist to ensure compliance with dosing volumes and the randomization schedule. After database lock, the weights will be recorded in the eCRF.*

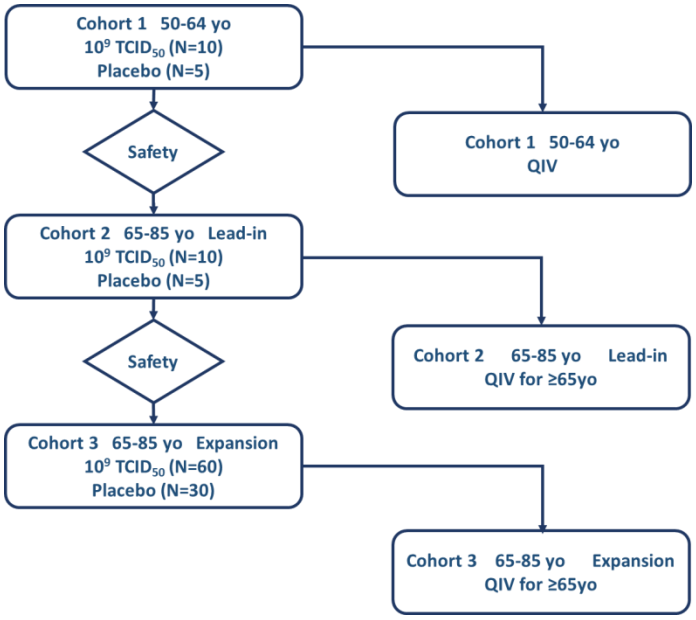
   Rationale: Language removed from the protocol as devices will not be weighed.

SYNOPSIS

Name of Sponsor/Company: FluGen Corporation, 597 Science Drive, Madison, WI 53711		
Name of Investigational Product: Sing2016 M2SR Vaccine		
Name of Active Ingredient: H3N2 Sing2016 (A/Singapore/INFIMH-16-0019/2016) M2SR monovalent influenza vaccine		
Protocol Number: FLUGEN-H3N2-V005	**Phase:** 1b	**Country:** US
Title of Study: Phase 1b clinical study to investigate the safety and immunogenicity of the H3N2 Sing2016 (A/Singapore/INFIMH-16-0019/2016) M2SR monovalent influenza vaccine in adults ages 50 to 85 years old.		
Studied period (years): Estimated date first subject enrolled: June 2021 Estimated date last subject completed: December 2021		
Objectives: **Primary:** • To assess the safety and tolerability of a single dose of monovalent Sing2016 H3N2 M2SR influenza vaccine delivered intranasally to healthy subjects 50 to 85 years of age **Secondary:** • To evaluate safety of inactivated quadrivalent influenza vaccine (QIV) when administered at least 28 days after receipt of investigational M2SR vaccine • To assess the immunogenicity (serum and mucosal antibody responses) of one administration of 10^9 fifty-percent tissue culture infective dose ($TCID_{50}$) Sing2016 M2SR vaccine Additional exploratory objectives may also be evaluated.		
Methodology: This is a randomized, double-blind, placebo-controlled Phase 1b study evaluating the safety and immunogenicity of the Sing2016 M2SR H3N2 influenza vaccine delivered intranasally to a healthy adult population aged 50 to 85 years. Eligible subjects will be randomized in a 2:1 ratio to receive one administration of 10^9 $TCID_{50}$ Sing2016 M2SR or placebo followed by a dose of licensed QIV at least 28 days later. Study enrollment will begin with a group of adults 50-64 years of age (Cohort 1, n=15) and will be followed by a lead-in group of subjects age 65-85 years of age (Cohort 2, n=15), followed by enrollment of an expansion group of subjects, ages 65-85 years of age (Cohort 3, n=90). Subjects will be enrolled into cohorts successively. Subject safety will be monitored by Investigators on an ongoing basis by daily review of electronic diary (eDiary) data and assessments during study visits. Study visits will preferably be conducted on-site in the clinic; however, due to the coronavirus disease of 2019 (Covid-19) pandemic, phone contacts to report solicited and/or unsolicited AEs and/or sample collection via a drive-through or remote laboratory will be allowed during the study, at the discretion of the Investigator. Prior to enrollment of Cohorts 2 and 3, a		

blinded review of available safety data from the prior cohort(s) will be conducted by the Medical Monitor and lead Principal Investigator (PI) after subjects have completed at least 7 days after investigational product (IP) treatment. If halting rules are met or if a safety concern requiring further assessment is identified, by either the MM or PI, then the review will be escalated to a Safety Review Committee (SRC), comprised at a minimum of two independent physicians experienced in vaccine research and a biostatistician. In addition, there will be 2 scheduled SRC meetings for informational safety updates. The first meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 1. The second meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 2. The safety reports generated for the SRC meeting are to be subjected to at least one round of review towards establishing a cleaned and QCed dataset. The purpose of these scheduled meetings is informational and not for gating decisions that determine progress to each successive cohort.

The study design is shown in the figure below:



An overview of the planned Study Cohorts is presented in the table below:

			Investigational Product (IP)		Licensed Quadrivalent Inactivated Influenza Vaccine (QIV)*
Cohort	Age Group (years)	n	10^9 $TCID_{50}$ Sing2016 M2SR	Placebo	
1	50-64	15	10	5	15
2	65-85	15	10	5	15
3	65-85	90	60	30	90
Total		**120**	**80**	**40**	**120**

* Subjects will receive a licensed QIV based upon availability of a US recommended and age-appropriate product that will be identified and provided by the Sponsor to all sites for dose administration at least 28 days after IP dose.

After subjects have signed an informed consent, they will be screened and assessed for inclusion/exclusion criteria including a test to evaluate serum antibodies to the Sing2016 hemagglutinin (HA) antigen. Within each cohort, subjects will be stratified based on a prescreening antibody titer to HA (by hemagglutinin inhibition [HAI] assay) from Sing2016 H3N2 influenza virus that is performed under a separate, site-specific protocol. That is, for each cohort ~50% of subjects will have an HAI titer of <40 and the remainder of subjects will have an HAI titer ≤320.

Eligible subjects will have a baseline blood draw, will be randomized, and will then receive either active Sing2016 M2SR vaccine or placebo on Day 1 at Visit 01. For 7 days after the dose of IP, the subject will record symptoms in an eDiary, the results of which will be monitored daily by the site for solicited and unsolicited adverse events (AEs). Subjects will return to the investigational site for follow-up on Days 8 and 29 (Visits 02 and 03, respectively). After at least 28 days (up to 42 days for Cohort 3) following administration of Sing2016 M2SR, licensed QIV will be administered at Visit 04. The interval between IP and QIV administration may be longer than 28 days for subjects who enroll in the study prior to early August 2021, based on the availability in the US of the licensed vaccine for the 2021-2022 Northern Hemisphere influenza season. QIV administrations may commence in September 2021 (as soon as the vaccine becomes available) and must be completed no later than early November 2021.

The final study visit (Visit 05) for each study subject will occur 28 days after licensed QIV administration. Serum samples for analysis of anti-HA antibody titers and nasal swabs will be collected as indicated in the Schedules of Events.

Serious adverse events (SAEs) occurring at any time during the study will be recorded. Administration site and solicited AEs will be recorded through 7 days after IP vaccination (V02). Unsolicited AEs will be recorded from time of consent through 28 days after both M2SR and QIV vaccinations.

While safety and tolerability are the primary objectives, this clinical study is also designed to assess the immune response to one dose of the investigational vaccine and after subsequent immunization with QIV. Immunogenicity will be assessed by measuring serum antibody responses by MN and/or HAI assay and mucosal antibody titers by ELISA. Additional immune parameters may be assessed including T cell responses. Subjects will be screened to determine HA titers against the target component (A/Singapore/INFIMH-16-0019/2016 [H3N2]) with the intent to enroll subjects with low titers (≤320 HAI titers).

Number of subjects (planned): Approximately 120 evaluable subjects

Diagnosis and main criteria for inclusion: Participating subjects will be healthy male and non-pregnant/lactating females who are 50-85 years of age. Enrolled study subjects may have certain stable chronic conditions based on study assessments and the clinical judgement of the Investigator and must be willing to adhere to the requirements of the study. Women of child-bearing potential must agree to abstain from sexual intercourse or to correctly use an acceptable method of contraception from 30 days prior to vaccination until 30 days after the last vaccination.

Subjects are not eligible for the study if they have acute or chronic physical or mental health conditions that would limit their ability to complete the study, result in increased risk, or interfere with the interpretation of study safety or immune response results based on assessment by the Investigator; have abnormal laboratory values according to protocol-specified criteria; who are receiving prohibited medications according to the protocol; who were vaccinated against influenza or had a flu-like illness within the last 6 months; who tested positive for human immunodeficiency virus (HIV), hepatitis C, or hepatitis B; who had a life-threatening reaction after a previous

administration of any vaccine or a history of allergy/hypersensitivity to any vaccine component; who had a recent (within 14 days) known exposure to Covid-19; or HAI titer >320 against Sing2016 H3N2 influenza.
Investigational product, dosage and mode of administration: The IP, Sing2016 M2SR, is a recombinant monovalent H3N2 influenza A virus. On Day 1, subjects will receive a single intranasal dose of IP vaccine or saline placebo. Subjects will be in a semi-recumbent position and receive up to 0.5 mL volume per nare (total maximum 1 mL) by employing two spray devices. Subjects will remain semi-recumbent and under direct observation for 30 minutes and will not be allowed to blow their noses, eat, or drink during this time.
Duration of treatment: Randomized subjects will receive one dose of experimental Sing2016 M2SR vaccine or placebo on Day 1 and a single dose of US-recommended and age-appropriate licensed QIV at least 28 days later. Duration of participation for each subject, including screening, is anticipated to be not less than 56 days and may be up to approximately 8 months.
Reference therapy, dosage and mode of administration: The reference product (placebo) is a physiological saline suitable for intranasal delivery. In addition, the following licensed vaccines will be used in this study: • A standard QIV in a suspension for injection. The QIV formulation will contain HA from 4 influenza strains representing A/H3N2, A/H1N1 and both the Yamagata and Victoria B lineages and is recommended for people 6 months of age and older. • A QIV recommended for people 65 years and older. For licensed vaccine administration, subjects will receive a single intramuscular injection of age-appropriate (50-64 years vs ≥65 years of age) QIV vaccine at least 28 days (up to ~42 days for Cohort 3) after IP administration. Licensed vaccines will be selected and supplied by the Sponsor so that all subjects, within an age cohort, receive the same vaccine product.
Criteria for evaluation: **Safety:** Safety will be assessed through an evaluation of AEs, SAEs, symptoms collection by eDiary, physical examination findings, and vital signs. **Immunogenicity:** Immunogenicity evaluations will include serum and mucosal antibody assessments.
Statistical methods: <u>Sample Size Justification</u> The sample size of 15 subjects each in Cohorts 1 and 2 and 90 subjects in Cohort 3 was chosen based on reports of studies of other vaccines in early-stage development and provides a safety assessment set prior to enrollment of additional individuals in Cohort 3. This study is not powered for any formal comparisons. <u>Analysis Datasets</u> The safety set will include any subject who receives administration of IP. The full analysis set (FAS) will include all subjects who are randomized, received IP and had at least one post baseline assessment. A per protocol set (PPS) may also be evaluated, which will include all subjects in the FAS with no major protocol violations or deviations. <u>Safety Analyses</u>

Safety and tolerability of the Sing2016 M2SR influenza vaccine alone, of the licensed QIV alone, and Sing2016 M2SR influenza vaccine when followed by a licensed vaccine as assessed by Investigator review of eDiaries (after IP administration only), solicited and unsolicited AEs, treatment-related AEs, SAEs, vital signs, and physical examinations.

Vaccine exposure will be summarized by actual dose, percent of expected dose, and percent of subjects receiving the expected dose.

Treatment-emergent AEs (local and systemic) will be summarized as N (%) with AEs, N (%) with related-AEs, and by worst severity. Incidence of treatment-emergent AEs (TEAEs) will be reported by Medical Dictionary for Regulatory Activities (MedDRA)-coded system organ class (SOC), preferred term (PT) and maximum severity. Incidence of most frequent TEAEs and most frequent treatment-related TEAEs will be reported by SOC and PT. Listings of subjects who died, discontinued the study due to an AE, or experienced an SAE will be provided. Dose-site reactions will be presented by reaction and severity. Post-administration symptoms (eDiary for post-IP) will be summarized by time point, worst occurrence, and days with symptoms.

Vital sign results and change from baseline results will be summarized by timepoint using descriptive statistics. Abnormal findings in physical examination will be listed.

Immunogenicity

Serum antibody and mucosal antibody responses will be summarized using descriptive statistics by sampling timepoint for all subjects and by baseline values. Seroconversion rates will be presented by timepoint and by baseline starting values.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
ACIP	Advisory Committee on Immunization Practices
AE	Adverse event
BMI	Body mass index
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CMI	Cell-mediated immunity
Covid-19	Coronavirus disease of 2019
CSR	Clinical study report
DMID	Division of Microbiology and Infectious Diseases
EDC	Electronic data capture
eDiary	Electronic diary
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
eTMF	Electronic trial master file
EUA	Emergency use authorization
FAS	Full analysis set
FDA	Food and Drug Administration
FTIH	First time in human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HA	Hemagglutinin
HAI	Hemagglutination inhibition
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IB	Investigator’s Brochure
ICF	Informed consent form
ICH	International Council on Harmonization

Abbreviation	Explanation
ID	Identification
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IIV	Inactivated influenza vaccine
IN	Intranasal
IP	Investigational product
IRB	Institutional Review Board
LPLV	Last patient last visit
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NA	Neuraminidase
NAI	Neuraminidase inhibition
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NSAIDS	Non-steroidal anti-inflammatory drugs
OTC	Over the counter
PBMC	Peripheral blood mononuclear cells
PCP	Phencyclidine
PI	Principal Investigator
PCR	Polymerase chain reaction
PPS	Per protocol set
PT	Preferred term
QIV	Quadrivalent (Inactivated) Influenza Vaccine
RBC	Red blood cells
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOC	System organ class
SOP	Standard operating procedure
SPG	Sucrose phosphate glutamate
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction

Abbreviation	**Explanation**
$TCID_{50}$	50% tissue culture infective dose
TEAE	Treatment-emergent adverse event
TIV	Trivalent (Inactivated) Influenza Vaccine
US	United States
USP	United States Pharmacopeia
VE	Vaccine effectiveness
WBC	White blood cells

1. INTRODUCTION

1.1. Overview

FluGen, Inc. is developing a novel M2 deficient single replication live influenza vaccine platform M2SR to provide safe, effective protection against seasonal influenza strains. The vaccine virus does not express an essential viral protein (influenza M2), restricting it to a single replication cycle in the host. The initial (prototypical) investigational monovalent agent, designated as Bris10 M2SR vaccine, was designed to elicit an immune response to the vaccine virus, influenza A/Brisbane/10/2007 (H3N2). Available data from Phase 1, Phase 1b and 2a studies in healthy adults 18-55 years of age have shown that a single intranasal dose of the prototype Bris10 M2SR vaccine is safe, immunogenic, and protects against a highly drifted H3N2 influenza challenge agent among subjects who demonstrated a serum immune response following vaccination. An updated vaccine, Sing2016 M2SR, encodes for the hemagglutinin (HA) and neuraminidase (NA) of A/Singapore/INFIMH-16-0019/2016, the H3N2 strain recommended for the 2018-2019 influenza season. In addition to updated HA and NA antigens, the vaccine backbone was modified to permit higher titers in production cells. Data from a Phase 1b study in healthy adults, ages 18-49 years, have shown that 1 or 2 doses of up to 10^9 fifty percent tissue culture infective dose ($TCID_{50}$) Sing2016 M2SR vaccine given at a 28-day interval were also safe and immunogenic. Ultimately, the vaccine development program aims to evaluate a quadrivalent (QIV) M2SR vaccine product containing two influenza A subtypes (H1N1-like and H3N2-like viruses) and two influenza B types.

1.2. Background Information

1.2.1. Influenza

Influenza viruses typically circulate widely in the United States (US) annually from the late fall through early spring. Although most persons who become infected will recover without sequelae, influenza can cause serious illness and death, particularly among persons age ≥ 65 years, children < 2 years, and individuals with underlying medical conditions that confer an increased risk of influenza-related complications (Grohskopf 2013). Over the past 10 years, estimated influenza-associated deaths have ranged from 12,000 to 61,000 annually, with 140,000 to 810,000 hospitalizations in the US annually (CDC 2020).

Annual influenza vaccination has long remained the primary means of preventing influenza infection and its complications and has been consistently recommended by various advisory bodies over the past 60 years. Although routine vaccination was initially recommended only for high-risk populations in the US, the Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP) have recommended since 2010 that all persons aged ≥ 6 months who do not have contraindications should be vaccinated (Grohskopf 2015). Vaccination is particularly important for people who are at high risk of serious complications from influenza, such as those 65 years of age and older. While vaccination with licensed vaccine can continue to be offered throughout the influenza season, the CDC recommends that individuals be vaccinated by the end of October each year (Grohskopf 2019).

Recent evaluations conducted by a US government-supported network of large clinics (US Flu-VE Network; Treanor 2012), have demonstrated an overall vaccine effectiveness (VE) of at

most 60% (95% confidence interval [CI], 53-66) for prevention of laboratory-confirmed, medically attended influenza illness due to any influenza virus, even in years in which there was a good match between vaccine and circulating strains (Treanor 2012). VE estimates in the US among individuals age ≥ 65 years have been consistently lower than in younger age groups, which is consistent with the well-documented reduction in immunogenicity of influenza vaccine in older adults (Osterholm 2012). VE has been as low as 9% in healthy older adults and even lower in older adults with underlying chronic diseases who are at risk for disability, hospitalization, and death. For H3N2 influenza strains, the VE in the >65-year-old age group was between 10-20% for the last five influenza seasons. Given the substantial and further increasing resource allocations to the US influenza vaccination programs, the continued search for more effective vaccines to further improve the population-level impact of the program – and particularly among older adults and others at high risk of serious influenza complications – remains a critically important public health challenge.

1.2.2. Current Influenza Vaccines

Currently available inactivated and recombinant influenza vaccines primarily aim to induce neutralizing antibodies that recognize the virus envelope protein HA. These antibodies can provide effective immunity, but they depend on a close match between the vaccine immunogen and circulating viruses. Such vaccines are therefore relatively ineffective against newly emerging viruses or viruses that have drifted away from the vaccine strain. Conventional egg-derived inactivated vaccines, which currently comprise approximately 90% of the US market, generally provide limited or no broadly cross-reactive (heterosubtypic) immunity needed to protect against divergent strains. Currently available trivalent (TIV) or quadrivalent (QIV) preparations provide the HA antigen and either none or limited and non-standardized amounts NA antigen. Moreover, at least one HA component of these vaccines must often be updated due to accumulated point mutations in the HA protein that allow the viruses to evade the human immune response. In addition, while mucosal and T cell immune responses have been associated with a reduction in influenza illness severity in subjects seronegative for influenza virus-specific antibody, these immune responses are generally not elicited following vaccination with conventional licensed inactivated vaccines (Wilkinson 2012; Clements 1986, Treanor 1999; Belshe 2000).

1.2.3. Live attenuated vaccines

In contrast to conventional inactivated and recombinant vaccines, live influenza virus vaccines are generally believed to offer broad-spectrum immune responses because they induce diverse types of adaptive responses, including serum antibodies, mucosal immunity, and induction of cytotoxic T lymphocytes, which target conserved virus epitopes (Clements 1986; Tamura 2005; Zhu 2010; Lanthier 2011; Ambrose 2012; Jin 2015). Only one such live vaccine, (FluMist®), which has been attenuated empirically by cold-adaptation, has been approved in the US, and is presently indicated only for persons 2-49 years of age. However, immune mechanisms conferring protection against influenza following receipt of FluMist vaccine are not fully understood; serum antibodies, mucosal antibodies, and influenza-specific T cells may play a role. Agreement has not yet been reached on a surrogate of efficacy for FluMist. Accumulating data also indicate that pre-existing cross-reactive immunity present in most adults limits live, attenuated vaccine virus replication, which in turn reduces a consistently effective immune

response and poor vaccine efficacy relative to inactivated influenza vaccine (IIV) (Eick 2009; Ohmit 2006; Wang 2009; Monto 2009). This in part explains the observation that young, seronegative children are the population in which FluMist vaccination is typically most effective.

1.2.4. FluGen's H3N2 (A/Singapore/INFIMH-16-0019/2016) M2SR vaccine

FluGen is developing a novel live virus vaccine platform known as "M2SR" to address the need for more effective influenza vaccines that will provide more consistent and broad-spectrum immunity for adults, and especially the elderly. The product is a live virus vaccine for intranasal administration that has been shown to provide protection against multiple influenza A subtypes in animal models (see below). The vaccine is a replication-defective recombinant influenza virus that does not express the essential M2 protein (and hence, the derivation of the "M2SR" naming convention [Single Replication phenotype due to lack of M2]). The M2SR vaccine virus backbone is comprised of (1) five of the six internal proteins of the donor virus Influenza A/Puerto Rico/8/34 (PR8), a strain that has been used for decades in traditional inactivated influenza vaccine manufacturing; (2) the sixth internal protein, M2, which is acquired from the cell substrate used to grow the vaccine virus, M2 Vero cells, which stably express and supply M2 protein for vaccine virus growth; and (3) the two influenza virus surface protein antigens, HA and NA, which can be derived from any selected Type A influenza strain. The resulting vaccine virus can infect normal cells in the respiratory epithelium of the vaccine recipient and then uncoat and initiate infection similar to a wild-type influenza virus, thereby evoking an immune response. However, because the M2SR genome does not encode for M2 protein, no viral progeny is subsequently produced after the initial (single round) infection, such that no cell-to-cell spread or subsequent shedding of virus occurs.

Sing2016 M2SR is a recombinant, monovalent influenza A virus that contains an Influenza A/Puerto Rico/8/34 (PR8) backbone with nine amino acid changes distributed over five genes (PB1, PB2, PA, NP and NS1) that facilitate growth in the production cell line, M2VeroA. The mechanism of attenuation (deletion of the M2 gene) is not affected. The Sing2016 M2SR also provides the HA and NA components from A/Singapore/INFIMH-16-0019/2016 that were recommended for the 2018-2019 influenza season.

1.3. Clinical Experience

Previous clinical experience with the monovalent H3N2 M2SR vaccine platform includes a First Time in Human (FTIH) Phase 1 dose escalation study in healthy adults, a Phase 2a challenge study in healthy adults, a Phase 1b dose escalation study in healthy adults, and a recently completed Phase 1 safety and immunogenicity study in adolescents (9-17 years old). Details on design and conduct of these studies are provided in the Investigator's Brochure (IB). All studies are complete, and data are unblinded for the Phase 1a FTIH, Phase 2 challenge, and Phase 1b dose escalation studies. As of 31 January 2021, the Phase 1 adolescent study remains blinded as the database is being prepared for lock. A total of 410 adults and 43 adolescents have been enrolled in these studies to date.

In the Phase 1 FTIH study, 72 adults (24 subjects per dose level) received a single dose of Bris10 M2SR at levels of 10^6, 10^7 or 10^8 $TCID_{50}$ and 24 subjects received saline placebo. In the challenge study, 52 adults received a single 10^8 $TCID_{50}$ dose of Bris10 M2SR and 56 subjects received saline, with 48 M2SR and 51 placebo subjects subsequently challenged with a drifted

H3N2 strain. In the Phase 1b dose escalation study, 206 (of 250 targeted) adults received two doses of either placebo (~20% of enrollment) or Bris10 M2SR at 10^8 $TCID_{50}$ (~20%), Sing2016 M2SR in amounts of 10^8, $10^{8.5}$, or 10^9 $TCID_{50}$ (~60% of enrollment; 165 active treatment subjects total). In the Phase 1 study, 43 adolescents (out of 50 planned) received one intranasal administration of 10^8 $TCID_{50}$ dose of Bris10 M2SR vaccine or placebo, followed by one intramuscular administration of licensed QIV administered 3 months later.

In the Phase 1 FTIH and the Phase 2a challenge studies, a total of 76 adult subjects received the highest dose of Bris10 M2SR tested (10^8 $TCID_{50}$) with no serious adverse events (SAEs) or adverse events (AEs) or other observations that would halt these studies. In addition, in the challenge study, 48 of 52 subjects who received the live, single-replication Bris10 M2SR virus (single dose of 10^8 $TCID_{50}$) were subsequently challenged with a live, replicating influenza virus. A portion of those individuals experienced infection and symptoms after challenge, consistent with influenza (i.e., nasal edema, nasal dryness, nasal pain/irritation, nasal congestion, rhinorrhea), but at a frequency less than that observed among individuals in the same study who did not receive vaccine prior to challenge. Unblinded safety data from the FTIH, challenge and dose escalation studies show one and two doses of the vaccine to be well tolerated, with related AEs that were generally mild or moderate in severity. No additional safety concerns were identified after challenge in the Phase 2a study. Reviews of unblinded safety data from the studies in adult populations have not identified any safety concerns. Of the 43 adolescents enrolled in the blinded Phase 1 study, no safety concerns related to the M2SR vaccine have been reported.

1.4. Rationale for the Current Study

Currently available inactivated and recombinant influenza vaccines primarily aim to induce neutralizing antibodies that recognize mainly influenza HA and depend on a close match between the vaccine and circulating viruses, rendering them much less effective against drift viruses. In addition, while a strong relationship has been established between pre-existing T cell immunity and illness severity, inactivated vaccines do not elicit cellular immune responses and generally do not provide broadly cross-reactive (heterosubtypic) immunity needed to protect against divergent strains. Moreover, mucosal immune responses have also been associated with a reduction in influenza illness in experimental challenge models and with live influenza vaccines; however, mucosal antibody responses are not elicited by conventional inactivated vaccines. For example, the severity of the 2014-15 influenza season was due to mismatch between the circulating epidemic strain and the inactivated vaccine, resulting in VE of just 13%. In the 2018-2019 influenza season in the US, the inactivated vaccines displayed a VE estimate of only 9% (www.cdc.gov) against H3N2, again due to mismatch between the vaccine strain and the circulating seasonal H3N2 virus that had accumulated antigenic changes (i.e., “drifted”). These recent examples indicate the need for an influenza vaccine that can protect against seasonal influenza drift variants.

In contrast to currently licensed influenza vaccines, pre-clinical testing of M2SR vaccine has shown induction of broadly reactive immune responses against multiple influenza subtypes and heterosubtypic protection in animal models, including demonstration of robust systemic, cellular and mucosal immunogenicity, even in the presence of pre-existing anti-influenza immunity. In

a pre-clinical proof-of-concept study, Bris10 M2SR provided protection to ferrets against a drifted H3N2 challenge virus, A/Alaska/140/2015, a virus belonging to clade 3c.2a1.

Human clinical trial participants who received Bris10 M2SR (10^8 $TCID_{50}$ dose) in the Phase 1a FTIH and Phase 2a studies also demonstrated broad-spectrum immune responses. In the FTIH study, vaccine induced serum and mucosal antibody responses were cross-reactive against multiple antigenically different H3N2 viruses including recent strains that belonged to the same clade (3c.3b) as the challenge virus used in the Phase 2a study.

Results from the Phase 2a challenge study showed that a single dose of 10^8 $TCID_{50}$ M2SR vaccine was effective against challenge in subjects who responded to vaccination by mounting a serum humoral immune response. Subjects who demonstrated any increase from baseline in serum antibody response (~50% of the M2SR cohort) by microneutralization (MN) to the intranasal M2SR vaccine had lower viral load and influenza-like symptoms after challenge than placebo subjects. These results suggested that if higher dose levels of M2SR could further enhance the serum immune response to influenza, then efficacy could be provided for in a higher proportion of M2SR recipients. Thus, a subsequent Phase 1b study evaluated one and two administrations of investigational M2SR at dose levels up to 10-fold greater than the dose employed in the Phase 2a challenge study.

The follow-on Phase 1b study was conducted with 206 healthy adults ages 18–49 years to evaluate whether an increased dose or a two-dose regimen could increase the proportion of subjects who respond to the M2SR vaccine, as demonstrated with serum, mucosal, and cellular immune responses. An early review of the group unblinded data showed that the higher doses (up to 10^9 $TCID_{50}$) and two doses administered with a 28-day interval were safe and well-tolerated; no SAEs were reported and no halting rules were met. Preliminary immunology data demonstrated that a single administration of the higher dose indeed resulted in a significantly higher rate of response to the M2SR vaccine. Subjects who received the 10^9 $TCID_{50}$ dose of M2SR dose demonstrated 71% seroconversion rate (≥4-fold) for serum hemagglutinin (HA) antibody in contrast to 28% seroconversion among recipients of 10^8 $TCID_{50}$ (dose used in the phase 2a challenge study).

The purpose of this proposed, additional Phase 1b clinical study is to assess for the first time the safety, tolerability/reactogenicity, and immunogenicity of H3N2 monovalent M2SR vaccines in an older adult population. A group of 15 adults age 50-64 will be enrolled first to assess safety. The probability that an AE with prevalence of 20% or more would be detected in the 15 subjects is >95%. After a safety review, the study will enroll 105 adults ages 65 to 85 years of age. One dose of investigational vaccine will be given intranasally followed at least 28 days later by delivery of an age-appropriate licensed QIV that is a quadrivalent influenza vaccine (QIV) recommended for the 2021/2022 season. Licensed vaccines will be selected and supplied by the Sponsor so that all subjects, within an age cohort, receive the same vaccine product. The same Sing2016 M2SR vaccine that will be used in this study has previously been investigated in adults 18-49 years of age, as described above. Demonstration of broadly reactive responses in the older adult population, similar to those found to be protective against challenge in adults, would support further clinical development of the M2SR vaccine platform in this age group.

Consistent with CDC recommendations, all subjects participating in the study will receive the licensed influenza vaccine at least 28 days after administration of study investigational product

(IP). The timing of the administration of licensed vaccine also will depend upon the availability of licensed vaccine, which usually occurs by early September each season. It is anticipated that the window between IP and QIV administrations for subjects in Cohorts 1 and 2 will be more variable, but no less than 28 days. The first cohort is anticipated to receive the first dose of IP in June 2021, but QIV may not be available until September. The window for administration of licensed QIV to study participants in Cohort 3 will be 28 to 42 days after intranasal administration of M2SR. Subjects will be followed for AEs occurring within 28 days after intramuscular inoculation with the licensed product. Subject diaries will not be employed after licensed vaccine administration.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

The primary objective of the study is to assess the safety and tolerability of a single dose of monovalent Sing2016 H3N2 M2SR influenza vaccine delivered intranasally to healthy subjects 50 to 85 years of age.

2.1.2. Secondary Objectives

The secondary objectives are:

- To evaluate safety of QIV when administered at least 28 days after receipt of investigational M2SR vaccine.
- To assess the immunogenicity (serum and mucosal antibody responses) of one administration of 10^9 $TCID_{50}$ Sing2016 M2SR vaccine.

2.1.3. Exploratory Objectives

The exploratory objectives are:

- To evaluate cell-mediated immune responses after IP administration.
- To evaluate additional vaccine-related immune responses (e.g., innate immunity) during the period of study participation.
- To assess the immunogenicity (serum and mucosal antibody responses) after administration of licensed, seasonal QIV vaccine when administered at least 28 days after one administration of 10^9 $TCID_{50}$ Sing2016 M2SR vaccine.

2.2. Endpoints

2.2.1. Primary Endpoints

The primary endpoints are:

- The number and percentage of study participants who experience solicited local and systemic reactions during the 7 days after administration of investigational M2SR vaccine or placebo, as recorded through subject electronic diary (eDiary).
- The number and percentage of study participants who experience unsolicited AEs during the 28 days after administration of investigational M2SR vaccine or placebo and prior to administration of licensed QIV.
- The number and percentage of study participants who experience SAEs from the time of informed consent through 28 days after receipt of QIV.

2.2.2. Secondary Endpoints

The secondary endpoints are:

- The number and percentage of study participants who experience unsolicited AEs during the 28 days after administration of licensed QIV.
- The number and percentage of study participants who after administration of Sing2016 M2SR or placebo and before administration of QIV demonstrate:
  - Influenza-specific serum antibody responses measured in MN and hemagglutination inhibition (HAI) assays at specified time points.
  - Influenza-specific mucosal immunoglobulin A (IgA) antibody responses measured in enzyme-linked immunosorbent assay (ELISA) assays at specified time points.

2.2.3. Exploratory Endpoints

The exploratory endpoints are:

- The number and percentage of study participants who after administration of Sing2016 M2SR or placebo demonstrate influenza-specific cellular immune responses measured by cytokine enzyme-linked immunospot (ELISPOT) and/or flow cytometry at specified time points.
- Additional influenza and/or vaccine-related immunological assays may be performed such as influenza-specific serum antibody responses by neuraminidase inhibition (NAI) and/or ELISA.
- The number and percentage of study participants who after administration of QIV demonstrate:
  - Influenza-specific serum antibody responses to each of the 4 vaccine components present in the QIV and to the experimental vaccine H3N2 as measured in assays such as MN, HAI, NAI and/or ELISA assays at specified time points.
  - Influenza-specific mucosal IgA antibody responses to each of the 4 vaccine components present in the QIV and to the experimental vaccine H3N2 as measured in ELISA assays at specified time points.
- Additional immunological assays may be performed against vaccine and non-vaccine related influenza strains after IP, placebo or IIV to evaluate:
  - GMT and GMFR at baseline and 28 days post-vaccination.
  - Number and percentage of study participants who demonstrate 2-fold and 4-fold rise from baseline.

3. STUDY DESIGN

3.1. Overview of Study Design

This is a randomized, double-blind, placebo-controlled Phase 1b study evaluating the safety and immunogenicity of the Sing2016 M2SR H3N2 influenza vaccine delivered intranasally to a healthy adult population age 50 to 85 years. Eligible subjects will be randomized in a 2:1 ratio to receive one administration of 10^9 $TCID_{50}$ Sing2016 M2SR or placebo followed by a dose of licensed QIV at least 28 days later. A group of adults 50-64 years of age (Cohort 1, n=15) will be followed by a lead-in group of subjects age 65-85 years of age (Cohort 2, n=15), followed by enrollment of an expansion group of subjects, ages 65-85 years of age (Cohort 3, n=90) (Figure 1). Subjects will be enrolled into cohorts successively.

Subject safety will be monitored by Investigators on an ongoing basis by daily review of eDiary data and assessments during study visits. Study visits will preferably be conducted on-site in the clinic; however, due to the coronavirus disease of 2019 (Covid-19) pandemic, phone contacts to report solicited and unsolicited AEs and/or sample collection via a drive-through or remote laboratory will be allowed during the study, at the discretion of the Investigator. Prior to enrollment of Cohorts 2 and 3, a blinded review of available safety data from the prior cohort will be conducted by the Medical Monitor and lead Principal Investigator (PI) after subjects have completed at least 7 days after IP treatment. If halting rules are met or if a safety concern requiring further assessment is identified, by either the MM or PI, then the review will be escalated to a Safety Review Committee (SRC), comprised at a minimum of two independent physicians experienced in vaccine research and a biostatistician.

In addition, there will be 2 scheduled SRC meetings for informational safety updates. The first meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 1. The second meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 2. The safety reports generated for the SRC meeting are to be subjected to at least one round of review towards establishing a cleaned and QCed dataset. The purpose of these scheduled meetings is informational and not for gating decisions that determine progress to each successive cohort.

An overview of the planned Study Cohorts is presented in Table 1.

Figure 1: Study Design

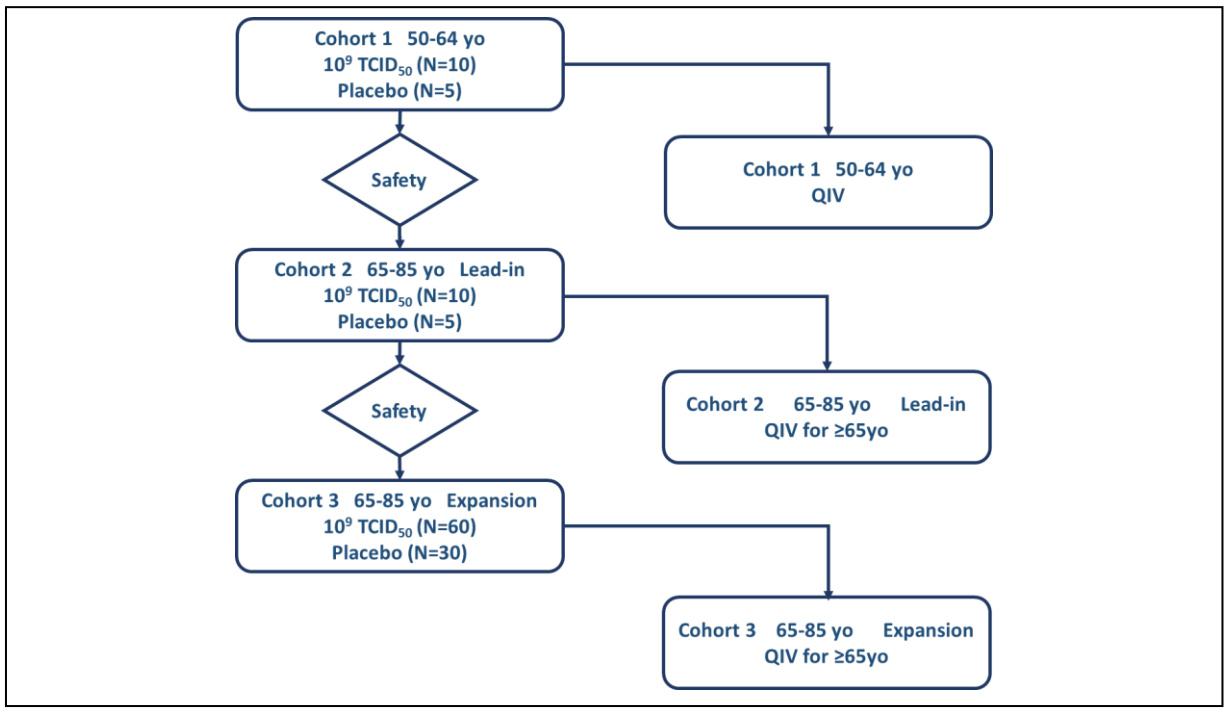

Table 1: Study Cohorts

			Investigational Product		Licensed Quadrivalent Inactivated Influenza Vaccine (QIV)*
Cohort	**Age Group (years)**	**N**	**10^9 $TCID_{50}$ Sing2016 M2SR**	**Placebo**	
1	50-64	15	10	5	15
2	65-85	15	10	5	15
3	65-85	90	60	30	90
Total		**120**	**80**	**40**	**120**

* Subjects will receive a licensed QIV based upon availability of a US recommended and age-appropriate product that will be identified and provided by the Sponsor to all sites for dose administration at least 28 days after IP dose.

After subjects have signed an informed consent, they will be screened and assessed for inclusion/exclusion criteria including a test to evaluate serum antibodies to the Sing2016 HA antigen. Within each cohort, subjects will be stratified based on a prescreening antibody titer to HA (obtained by HAI assay under a separate site protocol and ICF) from Sing2016 H3N2 influenza virus that is performed under a separate, site-specific protocol. That is, for each cohort ~50% of subjects will have an HAI titer of <40 and the remainder of subjects will have an HAI titer ≤320.

Eligible subjects will have a baseline blood draw, will be randomized, and will then receive either active Sing2016 M2SR vaccine or placebo on Day 1 at Visit 01. For 7 days after the dose of IP, each subject will record symptoms in an eDiary, the results of which will be monitored daily by the investigator for solicited and unsolicited AEs. Subjects will return to the

investigational site for follow-up on Days 8 and 29 (Visits 02 and 03, respectively). After at least 28 days (and up to 42 days for Cohort 3) following administration of Sing2016 M2SR, licensed QIV will be administered at Visit 04. The interval between IP and QIV administration may be longer than 28 days for subjects who enroll in the study prior to early August 2021, based on the availability of the licensed vaccine for the 2021-2022 Northern Hemisphere influenza season. QIV administrations may commence in September 2021 (as soon as the vaccine becomes available) and must be completed no later than early November 2021.

The final study visit (Visit 05) for each study subject will occur 28 days after licensed QIV administration. Serum samples for analysis of anti-HA antibody titers and nasal swabs will be collected has indicated in Table 2 (IP administration) and Table 3 (QIV administration).

SAEs occurring at any time during the study will be recorded. Administration site and solicited AEs will be recorded through 7 days after IP vaccination (Day 8). Unsolicited AEs will be recorded from time of consent through 28 days after both M2SR and QIV vaccinations.

While safety and tolerability are the primary objectives, this clinical study is also designed to assess the immune response to one dose of the investigational vaccine and after subsequent immunization with QIV. Immunogenicity will be assessed by measuring serum antibody responses by MN and/or HAI assay and mucosal antibody titers by ELISA. Additional immune parameters will be assessed including T-cell responses. Subjects will be screened to determine HA titers against the target component (A/Singapore/INFIMH-16-0019/2016 [H3N2]) with the intent to enroll subjects with low titers (≤320 HAI titer).

3.2. Number of Subjects

Approximately 120 subjects will be enrolled with approximately 80 subjects receiving study Sing2016 M2SR vaccine and 40 subjects receiving placebo.

3.3. Treatment Assignment

Eligible subjects will be randomized to study vaccine or placebo in a 2:1 ratio. Subjects will be stratified by HAI titer.

3.4. Expected Duration of a Subject's Participation in the Study

Duration of participation, including screening, for each subject is anticipated to be not less than 56 days and may be up to approximately 8 months.

3.5. Total Study Duration

It is anticipated that enrollment and follow-up will be completed within 10 months.

3.6. Discussion of Study Design

3.6.1. Rationale for Route of Administration

The Sing2016 M2SR vaccine contains live, single-replication virus. Intranasal administration of this vaccine, as proposed for this trial, mimics the natural route of infection of a wild-type influenza virus in which the virus adsorbs onto cells in the upper respiratory tract, enters the cell

and uses host cell processes to generate viral ribonucleic acid (RNA) and proteins and stimulate mucosal and systemic immune responses by the host.

3.6.2. Rationale for Dose Selection

In a Phase 1b dose-ranging study, the highest dose of Sing2016 M2SR vaccine studied (10^9 $TCID_{50}$) was well tolerated. No SAEs were reported and no halting rules were met. A single administration of this dose resulted in a significantly higher rate of immunological response to the M2SR vaccine compared with lower doses.

3.6.3. Rationale for Placebo Control

Placebo control will be used to establish the frequency and magnitude of changes in laboratory and clinical endpoints that may occur in the absence of active vaccination and will serve as a comparator for vaccine safety.

3.6.4. Rationale for Randomization and Blinding

Randomization will be used to avoid bias in the assignment of subjects to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups.

Blinded treatment will be used to reduce potential bias during data collection and evaluation of clinical endpoints. Only the pharmacist and/or their designee preparing the treatment, the unblinded monitor and the unblinded statistician or computer programmer who prepares the randomization list are unblinded with respect to treatment. Unblinded individuals will be restricted from participating in other study procedures the interpretation of which could be impacted by their unblinded status, e.g., subject assessments.

3.6.5. Rationale for Administration of Licensed Influenza Vaccine

The CDC recommends that all persons without contraindications should be vaccinated with any licensed age-appropriate influenza vaccine each year, and vaccination is particularly important for people who are at high risk of serious complications from influenza, such as those 65 years of age and older. Sing2016 M2SR is an investigational recombinant, monovalent influenza A virus vaccine. To satisfy the CDC recommendations for annual licensed flu vaccination, particularly in elderly persons, age-appropriate (for subjects 50-64 versus those ≥65 years of age) licensed influenza vaccines will be administered at least 28 days after IP administration in this study.

4. SELECTION OF STUDY POPULATION

4.1. Subject Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for study participation:

1. Subjects must be willing and able to provide written informed consent.
2. Cohort 1: age 50-64 years at time of consent; Cohorts 2 and 3: age 65-85 years at time of consent.
3. Subjects must be willing to adhere to the requirements of the study and willing and able to communicate with the Investigator and understand the requirements of the study.
4. Healthy adults and those with stable chronic conditions as determined by medical history, physical examination, vital signs, clinical safety laboratory examinations and clinical judgment of the Investigator to be eligible for study inclusion.
5. Negative test (urine) for drugs of abuse at screening. Subjects who are on stable (6 months or longer) medications prescribed by their physician which result in a positive screen for that substance are allowed at the discretion of the Investigator if there is no anticipated interference with study safety assessments.
6. Non-smoker (defined as no use of tobacco products and no use of inhaled non-tobacco products in the past 30 days prior to Study Day 1; nicotine patches and gum are allowed). Subject must also agree to restrict the use of these products until 7 days after last vaccine administration.
7. Women of child-bearing potential must agree to abstain from sexual intercourse or to correctly use an acceptable method of contraception, as described below, from 30 days prior to vaccination until 30 days after the last study vaccination.
8. Childbearing potential is defined by the occurrence of menses within the past year, and not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure and still menstruating.

Acceptable methods of contraception for female study participants include:

- non-male sexual relationships,
- abstinence from sexual intercourse with a male partner,
- monogamous relationship with a vasectomized partner,
- and correct use of
    - male condoms with the use of applied spermicide,
    - intrauterine devices,
    - NuvaRing®,
    - and licensed hormonal methods such as implants, injectables, contraceptive patches or oral contraceptives (“the pill”).

9. Women of childbearing potential must have a negative urine pregnancy test within 24 hours prior to vaccination with investigational product.

4.2. Subject Exclusion Criteria

1. Any subject who is a family member of a) study site personnel and other personnel directly involved in conduct or monitoring of study, or b) the Sponsor.
2. Any acute or chronic physical or mental health condition that would limit the subject's ability to complete the study, increase risk of study participation or participant, or may interfere with interpretation of study results as based on the assessment by the Investigator (e.g., a recent surgical procedure or planned procedure during the course of the study; other medical conditions, such as, for example, arthritis/rheumatoid arthritis, Guillain-Barré Syndrome, or immune suppression conditions, neuroinflammatory conditions, mental illness [including depression]; active hematological, renal, hepatic, pulmonary, central nervous, neurological, cardiovascular, endocrine [including poorly controlled diabetes mellitus] or gastrointestinal disorders).
3. Abnormal screening hematology or chemistry value per the US Food and Drug Administration (FDA) Guidance: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (Appendix 4). Note: Individuals with stable, Grade 1 laboratory abnormalities may be enrolled, if those lab results are not considered clinically significant by the Investigator and the individual otherwise meets study eligibility criteria.
4. Currently receiving, or planned to receive during the study, any immunosuppressive therapy. Any recent systemic corticosteroids must have been used for fewer than 2 weeks (14 days) and must be discontinued at least 1 month (28 days) prior to investigational product administration. Corticosteroids may be permitted if they are topical, intra-articular or intrabursal but not otherwise injected, inhaled or nebulized.
5. Has been vaccinated against influenza within the last 6 months (182 days) before investigational product administration.
6. Had a flu-like illness (i.e., fever, chills and myalgia), influenza treatment (i.e., commercial drug such as Oseltamivir), or prophylactic influenza viral drug administered in the previous 6 months before investigational product administration.
7. Positive test at screening for human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV).
8. Recent (within 14 days) known exposure to Covid-19 through a close contact as defined by CDC and/or confirmed Covid-19 infection within the past 3 months.
9. Experienced a life-threatening reaction(s) after a previous administration of any vaccine or experienced an allergic reaction after a previous administration of any influenza vaccine or component. In addition, a history of allergy/hypersensitivity to any vaccine (M2SR or licensed QIV) component (sucrose, sodium chloride, phosphate, glutamate, egg protein, formaldehyde, octylphenol ethoxylate [Triton®X-100]), or material in either the nasal or intramuscular delivery device (polycarbonate, polypropylene, synthetic rubber).

10. Females who are pregnant or nursing.
11. Non-vasectomized males with female partners of childbearing potential who are unwilling to use a highly effective form of contraception from the time of enrollment through at least 28 after administration of the investigational product. Males also may not donate sperm through at least 90 days after receiving the investigational product.
12. Blood disorder (easy bruising or bleeding) that may prolong bleeding and for which Investigator deems it would be ill-advised to administer an intramuscular vaccine.
13. History of receipt of any live virus vaccine within 56 days of study entry, licensed or investigational vaccine within 28 days of Visit 01 or investigational drug within the past six months. An exception is made for receipt of a Covid-19 vaccine whether licensed or under emergency use authorization (EUA) as long as the final dose was given at least 28 days prior to Visit 01.
14. Planned receipt of licensed vaccine, other than the study-provided licensed influenza vaccine, during the 28 days following Visit 01 or another investigational vaccine or investigational drug during the study period.
15. Receipt of blood/plasma products or immunoglobulin within 6 months before administration of the investigational product or planned for during the period of study participation.
16. Donation of blood or blood product of at least approximately 1 pint (~470mL) within 60 days of IP receipt or planned within 3 months (84 days) after IP administration.
17. Acute febrile illness within 72 hours prior to investigational product vaccination, defined as the presence of a moderate or severe illness with or without fever (as determined by the Investigator through medical history and physical examination), or presence of a fever >38ºC orally. Vaccination (or administration of the second dose of vaccine) should be delayed until the subject has recovered. Persons with a minor illness, such as diarrhea, or mild upper respiratory tract infection with or without low-grade fever, may be enrolled after resolution of the illness.
18. Resident of a nursing home or long-term care facility or having the need for coordinated nursing care or assistance with/monitoring of activities of daily living.
19. Subjects with HAI titer greater than 320 against Sing2016 H3N2 influenza.

4.3. Lifestyle Guidelines

No blood donation will be allowed within 3 months (84 days) after IP administration.

Information on prohibited therapies is available in Section 5.9.2.

4.3.1. Contraception

Female subjects are either postmenopausal (defined as without menses for at least 12 consecutive months without an alternative medical), surgically sterile, or if of childbearing potential, must agree to sexual abstinence or the use of a reliable method of contraception as noted in Section 4.1. Non-surgically sterile male subjects who are sexually active with a female

partner(s) of childbearing potential must also agree to use a reliable method of contraception as noted in Section 4.1.

Method of contraception will be captured on the appropriate data collection form.

5. TREATMENTS

5.1. Study Vaccine

Sing2016 M2SR Vaccine: The IP is termed Sing2016 M2SR and is a recombinant, monovalent influenza A virus that was initially generated by use of a plasmid rescue system. The vaccine virus backbone is composed of the 6 internal protein genes of the donor virus PR8 similar to those used in the prototype Bris10 M2SR vaccine used in the FTIH trial but with nine amino acid changes distributed over five genes (PB1, PB2, PA, NP and NS1) that facilitate growth in the production cell line, M2VeroA. The mechanism of attenuation (deletion of the M2 gene) is not affected. Since the virus does not express an essential viral protein (influenza M2), it is restricted to a single replication cycle in the host. The Sing2016 M2SR vaccine also encodes the viral antigens HA and NA from Influenza A/Singapore/INFIMH-16-0019/2016 that were recommended for the 2018-2019 influenza season.

A pharmacist or designee will thaw the vial contents to room temperature just prior to dose administration. The contents will be drawn into two 1 mL disposable polypropylene syringes each fitted with a mucosal atomization device (MAD300; Teleflex, Salt Lake City, Utah, US) for intranasal delivery. The barrel of the syringe will be wrapped with a label to mask any coloration of the contents that might otherwise unblind the site staff or subject.

5.2. Placebo Administered in the Study

The reference product (placebo) is a sterile, physiological saline suitable for intranasal delivery. The placebo will be provided as a liquid and in single-use vials. The placebo will be drawn into two 1 mL disposable syringes (~50% volume each) that are fitted with a Teleflex MAD300 sprayer for intranasal delivery. Administration and post-dose restrictions will be as described for the M2SR vaccines.

The placebo is a clear solution whereas the Sing2016 M2SR vaccine is a light yellowish color. The unblinded pharmacy staff will prepare the vaccine and placebo doses, fill delivery devices and apply an opaque label to the device barrel to obscure any coloration of the contents and maintain the blind.

Commercially available supplies of the placebo will be used and will be supplied by the site following approval from the Sponsor.

5.3. Licensed Vaccines Administered During the Study

For licensed vaccine delivery, subjects will receive a single intramuscular injection of licensed QIV deemed appropriate for their age at least 28 days after IP administration. Subjects will be in a seated position and receive an injection of the licensed vaccine delivered to the deltoid muscle.

The QIV will be supplied to the sites from a distributor engaged by the Sponsor.

5.4. Packaging and Labeling

5.4.1. Study Vaccine

Manufacturing, packaging, and labeling of Sing2016 M2SR vaccine is under the responsibility of the Sponsor.

The Sing2016 M2SR for this study was manufactured using master virus stocks and working cell bank at Ology Bioservices (Alachua, Florida, US) under current Good Manufacturing Practice in a liquid formulation. The Sing2016 M2SR vaccine will be provided frozen and in single-use cryovials at 2.2e9 $TCID_{50}$/mL. The vaccine is suspended in SPG-NaCl buffer comprised of 303 mM sucrose, 5 mM glutamic acid, 136.9 mM sodium chloride, 2.67 mM potassium chloride, 1.47 mM potassium dihydride phosphate and 8.1 mM disodium phosphate, at pH 7.2.

The primary package for the vaccine product is a 2 mL cryovial with a threaded cap closure. The vaccine will be labeled according to local law and regulatory requirements. Each vial label will include the following information: product name, item and lot numbers, date of manufacture, storage condition, volume, vial number, manufacturer, and "Caution: New Drug – Limited by Federal law to Investigational Use."

The vaccine vials will be packed into multi-vial boxes. Each box will be labeled with the following information: product name, item and lot numbers, storage condition, vial number range and volume, manufacturer and "Caution: New Drug – Limited by Federal law to Investigational Use."

5.4.2. Placebo

The placebo is a commercially available physiological saline that will be purchased by the site after approval from the Sponsor. The 0.9% sodium chloride for injection, US pharmacopeia (USP), is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. It is preservative-free and supplied in a single-dose container. The pharmacist or designee will aseptically fill placebo into the intranasal delivery devices on an as needed basis.

5.4.3. Licensed QIV

The licensed QIV will be purchased by the Sponsor through a distributor for provision to the sites.

The licensed vaccines will contain HA proteins from each of the 4 monovalent strains, i.e., two influenza A subtype viruses and two influenza type B viruses recommended for use in the 2021-2022 influenza season. The proteins are suspended in a sodium phosphate buffered isotonic sodium chloride solution and may contain trace amounts of egg protein, formaldehyde and Triton X-100. The vaccines are clear and slightly opalescent in color. They will be supplied in vials or pre-filled syringes, neither of which contain natural rubber latex. The pharmacist will shake the vial or syringe before administering the dose as an intramuscular injection.

5.5. Storage and Drug Accountability

The Investigator (or designee) is responsible for the safe storage of all study treatments assigned to the clinical site, in a locked, secure storage facility with access limited to those individuals authorized to dispense the study drugs and maintained within the appropriate ranges of temperature. All study drugs must be stored as specified at delivery and in the original packaging. Instructions for the storage and handling of the study drugs will also be provided to the clinical site.

5.5.1. Study Vaccine

The M2SR vaccine will be delivered frozen and must be stored frozen at < -65°C in the provided multi-vial boxes. Each vaccine vial is a single-use vial. Once the vial contents have been thawed, the vial must be stored at 2-8°C prior to use in vaccine administration. The thawed vial contents will remain stable in the Sponsor-identified cryovials, for up to 8 hours. Any vaccine product that has been thawed and held at 2-8°C for longer than 8 hours is no longer suitable for use in the study and must be accounted for. Such product will be labeled as damaged and will be quarantined at room temperature until the study monitor conducts on-site drug accountability. After that, this product should be destroyed per clinic Standard Operating Procedures (SOPs) and a certificate of destruction, or equivalent should be filed on site and provided to the Sponsor for internal filing.

Investigational product must be stored under temperature-controlled and monitored conditions. The site will also maintain a temperature log record of the relevant equipment for IP storage. Should an excursion in storage conditions occur, the clinical site must not further dispense the affected study vaccine until after notification and consultation with the Sponsor.

The Investigator is responsible throughout the study for ensuring inventory and account record of all study drugs received at the clinical site. All drug accountability records must be stored in the site file and must be readily available for inspection by the study monitor and/or auditor, and open to regulatory inspections at any time.

As misallocations of study drug may have a detrimental effect on subjects' safety and/or the study drug's efficacy and are a potential source of bias, utmost care should be taken to correctly dispense the study drugs as assigned by the randomization code.

The vaccine and placebo should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or by a hospital/clinic pharmacist or designee. The pharmacist or designee must maintain accurate records demonstrating date and amount of vaccine and the placebo supplied to whom and by who. The investigational vaccine and placebo will be supplied only to subjects participating in the study.

The Sponsor's designated study monitor will periodically check the supplies of vaccine and the placebo held by the Investigator or pharmacist/designee to ensure accountability and appropriate storage conditions of all vaccine and placebo used.

Unused vaccine and placebo must be available for verification by the study monitor during on-site monitoring visits. Any discrepancies between returned and expected returned vaccine and placebo should be explained and documented.

After the database has been locked and all on-site drug accountability has been completed by the monitor, any unused vaccine (including neat and diluted remnants) may be returned to the Sponsor or destroyed at the clinical site with the Sponsor's written permission (in this case a certificate of destruction or equivalent will be provided and filed in the electronic Trial Master File [eTMF]).

Hazardous materials such as used ampoules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

5.5.2. Placebo

Each placebo cartridge is a single-use container. The solution will be stored at USP controlled room temperature (20 – 25°C) prior to use in treatment administration. The contents may be used up to the date of expiry as stated by the manufacturer.

Procedures for handling and storage of the commercially available placebo are detailed on the product-specific labels.

5.5.3. Licensed QIV

The licensed QIV is supplied as vials or prefilled syringes without needles. The QIV will be stored refrigerated at 2-8°C. Do not freeze this vaccine. If product is frozen it must be discarded. The contents may be used up to the date of expiry as stated by the manufacturer.

5.6. Blinding

The blind will be maintained during this trial, including use of the following measures:

1. All subjects, whether receiving Sing2016 M2SR vaccine or placebo, will undergo the same procedures, i.e., administration, AE assessment, blood collections, nasal swabs, etc.
2. The unblinded pharmacist or designee will prepare doses (active and placebo), fill delivery devices and apply an opaque label to the device barrel to obscure any coloration of the contents.
3. The unblinded site staff and unblinded study monitor will agree to maintain the blind by not providing details of the dose (active or placebo) to any blinded clinic staff including the Investigator and to study subjects. Furthermore, the unblinded individuals will be restricted from participating in other study procedures the interpretation of which could be impacted by their unblinded status, such as evaluation of AEs.
4. If any blinded investigational site staff learn of treatment assignments, a record of the event will be maintained, and the Investigator will be informed. The Investigator will consult with FluGen on how to preserve the blind.
5. Labs supplied with subject samples for analyses will be blinded to treatment assignment.

Except for the unblinded statistician or computer programmer who generates the randomization list, the unblinded pharmacist(s) or their designee(s) who prepare the study product for administration and the unblinded study monitor who may observe the study product preparation and performs IP accountability, all subjects, the Investigator, statistical programmers and all

clinical site staff will be blinded to IP treatment (vaccine or placebo). All clinic staff who may be involved in making assessments of safety (including local reactions) will be blinded to treatment. Should a blinded monitor observe study product preparation, precautions must be taken to maintain the blind.

Masking of the IP (vaccine or placebo) will be described in the Pharmacy Manual.

The randomization schedule will be inaccessible to the Investigator or to the blinded study team (monitors and/or FluGen team) during the study. After the final study visit is complete for all subjects, after database lock, and after reconciliation with pharmacy and site records, the randomization code will be provided to the Study Statistician to enable data analysis by the Sponsor.

The unblinded Statistician will provide the randomization list to the unblinded pharmacist or their designee. This list will be used to prepare study product dosages and in the event of an emergency code break (see below).

In the event of a medical emergency wherein knowledge of the IP treatment assignment will influence the subject's care, the PI may be provided with the IP treatment assignment for that subject, at the discretion of the PI. The unblinded pharmacist or their designee will use the IP treatment assignment code to break the blind for the PI only in case of emergency. The PI will contact the FluGen Medical Monitor and document the event with the information regarding the reasons for unblinding as soon as possible, and no later than 24 hours after such unblinding. In the event the IP treatment code is broken, it will be broken ONLY for the subject in question. The reason for unblinding will be documented in the subject's paper source. Subjects who are unblinded for any reason during their participation in the study will not be replaced and will be withdrawn immediately from the study; however, all attempts will be made to collect safety data through 28 days post-dose of the vaccine/placebo. In the event of a request to break a treatment code, the study monitor will be notified within 24 hours and the rationale for breaking the code will be documented.

5.7. Dose and Administration

5.7.1. Investigational Vaccine

A rationale for the dose of Sing2016 M2SR vaccine selected in this study is provided in Section 3.6.2.

Subjects will be randomly and sequentially assigned, according to the randomization schedule and in a blinded fashion, to vaccine or placebo in a 2:1 ratio.

Each vaccine and placebo dose will be filled into two sterile 1 mL polypropylene syringes (HenkeJect) that are each fitted with a Teleflex MAD300 sprayer for intranasal delivery (Figure 2) by the unblinded pharmacist or designee.

Figure 2: Image of the MAD300 Sprayer Device Fitted to a Syringe

To fill the syringe, the contents are drawn into the syringe barrel via an 18-gauge needle. The needle is removed and replaced with the MAD300 sprayer via a Luer lock fitting. A sterile cap is fitted to the end of the sprayer to generate a closed system until time of dose administration. Further details for filling and actuating the spray device are provided in the Pharmacy Manual. Components for IP dosing will be supplied by the Sponsor. The pharmacist or designee will wrap an opaque label completely around the circumference of the syringe barrel to conceal any color of the liquid contents and will use an indelible marker to record the subject's identification (ID) on the label. The clinical staff credentialed to administer vaccines will then use the masked, filled spray device to administer the dose (active or placebo) intranasally to the subject.

For delivery, subjects will receive a single intranasal dose of vaccine (or placebo) via two MAD300 spray devices. Subjects will be in a semi-recumbent position and receive ~50% dose volume per nasal cavity by employing 2 spray devices. Subjects will remain semi-recumbent and under observation for approximately 30 minutes and will not be allowed to blow their noses, eat, or drink during this time; any deviations are captured in the source documents and reported to the study monitor/clinical project manager.

Any deviation from the dose defined in the protocol must be documented on source and in the electronic case report form (eCRF) system.

5.7.2. Licensed Influenza Vaccine

See package inserts for instruction on how to prepare and administer the licensed vaccine. At time of QIV administration, subjects who are younger than 65 years of age will receive a standard QIV and those who are at least 65 years of age will receive a QIV indicated for people over 65 years of age. The vaccines will be prepared and administered by the pharmacist or designee per instructions in the package insert.

5.8. Treatment Compliance

Monitoring treatment compliance is not applicable because treatment will be administered per protocol in the clinic by study personnel.

5.9. Prior and Concomitant Therapy

The use of concomitant therapies should be kept to a minimum throughout the study. All therapies administered (prescriptions and over-the-counter medications), other than the vaccine, from informed consent until the final study visit, must be recorded in the source documents and in the concomitant therapy section of the eCRF (name of the drug, dosage, route and dates of administration).

In the event that medical conditions after study treatment dictate use of medications, subjects are encouraged to obtain adequate care, comply with the course of therapy as prescribed by their physician, and inform the Investigator as soon as practical.

Subjects may receive a vaccine that is licensed or under an EUA if it is the opinion of the investigator that such treatment is appropriate and necessary. The preference, when possible, is to avoid such treatment during the 28 days post-visit 01.

The Sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered to a study subject.

Female subjects of childbearing potential and non-vasectomized male subjects having a female partner of childbearing potential must agree to the use of an effective method of contraception throughout the study, as outlined in Section 4.1. The use of oral, injectable, and implantable hormonal contraceptives is to be recorded in the source documents and in the concomitant therapy section of the eCRF.

5.9.1. Permitted Concomitant Therapies

The following medications and treatment are permitted in this study after Day 1:

- Non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, or antihistamines may be used after dosing if the subject has a fever ≥ 38.0°C (100.4°F) or if the subject has significant nasal pain, myalgia, or headache. NSAIDs or acetaminophen should not be taken prophylactically and in the event of use should be documented in the eDiary.
- Low-dose aspirin.
- Medications used by the subject to treat stable chronic health conditions, which were reported previously during the screening visit, if use of those medications does not interfere with assessments of study safety and immune responses.

5.9.2. Prohibited Concomitant Therapies

The following medications are not permitted in the study:

- Concomitant use of any new (not previously used by subject or dosage changed within past month) prescription (except for contraceptive pills, implants, transdermal or injections), new vitamins or new dietary supplements. Over-the-counter (OTC) medications are discouraged but allowed for 7 days prior to IP vaccination and continuing until 7 days after last vaccination. Any such use of OTC must be recorded in the source documents along with the associated AE (if applicable).
- Any licensed vaccine, other than the study-provided licensed influenza vaccine, during the 28 days following Visit 01 or another investigational vaccine or investigational drug during the study period.
- Receipt of blood/plasma products or immunoglobulin during the study period.

Any medication that is not covered by the permitted and non-permitted medication sections can be allowed at the discretion of the Investigator and documented on eCRF.

6. ASSESSMENTS

6.1. Timing of Assessments

An overview of the timing of treatments and assessments is given in the Schedules of Events (Section 6.1.1). The Schedule of Events and visits for IP administration is provided in Table 2 and the schedule and visits for QIV administration is provided in Table 3.

When visit assessments are planned at the same time point in the study, the order of assessments should be in order of less invasive to more invasive. For example, during the randomization visit, vitals, medical history, pregnancy testing and drug testing should be conducted before blood work and subsequent nasal swab.

Prior to all on-site visits, the site will assess the subject's health status in accordance with local policies to evaluate whether subject is fit to attend the clinic. This may include a phone call or email, etc. with questions regarding Covid-19-related symptoms, known exposure to Covid-19 within past 14 days and/or subject having confirmed positive test for Covid-19. A subject deemed not suitable should not attend the site and the PI or designee should contact the Medical Monitor to discuss subject study status.

6.1.1. Schedules of Events

Table 2: Schedule of Events – IP Administration

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**Early Term[5]**
Study Day Post-First Dose	**Days -28 to -1**	**Day 1**	**Day 8 ±1**	**Day 29 ±3**	
Informed Consent	X				
Inclusion/Exclusion	X	X[2]			
Demography	X				
Physical exam - including height/weight	X[1]				
Limited physical exam[7]		X[2,3]	X	X	X
Medical history	X	X[2]			
Vital signs (BP, HR & body temp)	X	X[2, 3]	X	X	X
Hematology & Chemistry[4]	X	[2, 10]			X[9]
Urinalysis including drugs of abuse	X				
Urine Pregnancy (females, child-bearing potential)	X	X[2]			X[9]
Testing for HIV, HBV, HCV	X				
Nasal swab – immunogenicity		X[2, 6]		X[6]	X[6]
Nasal swab - respiratory agent(s)	X[12]	X[2,13,14]	X[12,14]		
Serum collection (HAI, MN, NAI and ELISA)	X[11]			X	X
PBMC collection (50 mL for CMI) and plasma		X[2]		X	
Blood collection (5 mL for RNA[15])		X[2]	X		
Investigational dose administration		X			

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**Early Term[5]**
Study Day Post-First Dose	**Days -28 to -1**	**Day 1**	**Day 8 ±1**	**Day 29 ±3**	
Dispense & train on eDiary and thermometer use[8]		X	X		
Review eDiary data		←-------X------→			X
AEs/Con meds	X	X[2,3]	X	X	X
SAEs	X	X[2]	X	X	X

AE=adverse events; BP=blood pressure; CMI=cell-mediated immunity; Covid-19=coronavirus disease of 2019; ELISA=enzyme-linked immunosorbent assay; HAI=hemagglutination inhibition; HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; HR=heart rate; IP=investigational product; MN=microneutralization; NAI=neuraminidase inhibition; PBMC=peripheral blood mononuclear cells; PCR=polymerase chain reaction; PI=Principal Investigator; RNA=ribonucleic acid; SAE=serious adverse events; temp=temperature; term=termination; V=visit; WBC=white blood cell count.

1 Complete physical is required; neurological, rectal and gynecological exams do not need to be performed unless clinically indicated.

2 Prior to dosing.

3 At approximately 15 minutes after dosing.

4 Laboratory tests to establish baseline status as listed in Appendix 3 for hematology and biochemistry.

5 Early Term visit is applicable if a subject does not complete the study within 28 days after IP dose administration. eDiary to be reviewed, if within 7 days of dose administration.

6 Collect sample prior to swab for respiratory agents.

7 Specifically includes the nares (including edema), throat, and lungs (including evaluation for wheezing) up to and including Day 29. A targeted physical examination may be performed on other body systems if indicated based on review of interim medical history or subject symptoms as observed by site or by PI.

8 Dispense on day of dose then ongoing review for 7 additional days following vaccine administration using electronic capture and internet-based portal. If subject does not populate eDiary every 24-hours, the site will contact them for follow-up and document the contact in subject's source.

9 Sample collected if subject presents with AE requiring laboratory assessment.

10 If screening blood collection was greater than 28 days prior to Day 1 then laboratory assessments are to be repeated to establish baseline.

11 Serum at screening will be tested for HA immunology and will be considered baseline for the subject. A prescreen titer, collected under a separate site-specific protocol will be used to assess entry criteria.

12 PCR Test for Covid-19.

13 V01 sample to be tested by multiplex PCR test for abbreviated list of respiratory pathogens including Covid-19, Flu A, Flu B and RSV. Additional swabs may be collected at unscheduled visits, if the subject presents with respiratory symptoms that warrant additional testing; in such case a full respiratory panel is to be performed.

14 Collect, store, then batch test.

15 RNA analyzed for gene expression (no genetic testing of inherited diseases will be done)

Table 3: Schedule of Events – QIV Administration

Study Visit Number	**V04**[1]	**V05**
Study Day **Post-QIV Dose**	**Day 1**	**Day 29** **±7**
Safety check	X[2]	
Limited physical exam[3]	X[2,4]	X
Vital signs (BP, HR & temp)	X[2]	X
Nasal swab – immunogenicity	X[5]	X
Serum collection (HAI, MN, NAI and ELISA)	X[2]	X
Administer licensed influenza vaccine[6]	X	
AEs/Con meds	X[2]	X
SAEs	X[2]	X

AE=adverse events; BP=blood pressure; ELISA=enzyme-linked immunosorbent assay; HAI=hemagglutination inhibition; HR=heart rate; QIV= quadrivalent influenza vaccine; IP=investigational product; MN=microneutralization; NAI=neuraminidase inhibition; PI=Principal Investigator; QIV=quadrivalent inactivated vaccine; SAE=serious adverse events; temp=temperature; term=termination; V=visit.

1 V03 from IP dosing schedule and V04 from QIV dose schedule may be combined on same day if V04 occurs at least 28 days after V01. In such case, activities do not need to be duplicated during the visit.

2 Prior to dosing. Subjects will be assessed for any known reactions to licensed influenza vaccines.

3 Specifically includes the dermal injection site. A targeted physical examination may be performed on other body systems if indicated based on review of interim medical history or subject symptoms as observed by site or by PI.

4 At approximately 15 min after dosing.

5 Sample collected for secretory IgA immune response by ELISA

6 Licensed vaccine supplied to site by Sponsor; product used will be age-appropriate for the subject

6.1.2. Screening Period

In a private, individual setting, subjects will be given a full explanation of the nature of the study and written informed consent (Institutional Review Board [IRB] approval will be obtained before any study-related assessment) will be carried out. Group consenting will not be permitted and subjects requiring a Legally Authorized Representative may not participate in this study.

Screening for eligible and consenting subjects will be performed within 28 days prior to randomization/IP administration. Subjects will be re-confirmed for eligibility on Day 1 (V01) prior to dose administration. Chemistry and hematology at V00 will serve as eligibility and baseline values unless collected more than 28 days prior to V01. In such case the lab tests will be redone to establish baseline only.

Subjects will have been consented and prescreened for influenza serosusceptibility under a separate, site-specific IRB-approved informed consent form (ICF). The prescreening protocol and related ICF will be made available upon request by the Sponsor.

At screening, subjects will be asked to attend the clinical site to have assessments performed as indicated in the Schedule of Events (Table 2).

All results from the screening procedure needed to evaluate eligibility, including the clinical laboratory results, must be available prior to study vaccination on Day 1. Any abnormal assessment at the screening visit will be assessed according to its clinical relevance, and if found clinically significant, the subject will not be included in the study.

During the screening period, the following procedures and assessments will be carried out for each subject to determine their eligibility for participation in the study:

- Obtain informed consent prior to any procedures being performed
- Record demographics
- Confirm eligibility criteria for enrollment and vaccination
- Obtain medical history
- Record concomitant medications
- Perform complete physical examination (neurological, rectal and gynecological exams do not need to be performed unless clinically indicated), including height and weight
- Vital signs (blood pressure, heart rate, and body temperature)
- Collect urine for dipstick pregnancy test (applicable females only)
- Collect nasal swab for Covid-19 testing
- Collect blood for serology for HIV, HBV and HCV
- Collect blood for serum chemistry and hematology
- Collect blood for immunogenicity assays
- Urinalysis (dipstick) and urine screen for drugs of abuse
- Record any concomitant therapy/medically attended AEs/SAEs after signing of consent

Beginning at the screening visit and at subsequent visits subjects will be instructed on Covid-19 best practices including wearing a mask in public spaces whether indoors or outdoors, distancing from people outside of their household, frequent washing of hands, etc. At each visit, subjects will be asked a series of scripted questions to evaluate their exposure to Covid-19 and results will be documented in the source.

Unscheduled visits may be planned (up to Day 28) to assess, confirm, and follow-up on out-of-range clinical laboratory test, or vital sign values that determine a subject's eligibility, or in case of a positive urine drug screen. The result of the retest will be considered for subject eligibility. Findings made during unscheduled visits should be reported in the eCRF system.

A record of the number of screening failures and the reasons for screening failure will be captured in a study file. Data for subjects who fail screening or who are not randomized into the study will not be included in the study database. The failure details will be documented in a study file maintained by the site.

6.1.3. Licensed Vaccine Visit

Subjects will undergo a safety check prior to dose administration of the licensed influenza vaccine product to confirm there are no contraindications to the licensed vaccine as per the package insert. Subjects will be excluded from dosing if they have ever had a severe allergic reaction to eggs or egg products or after getting any influenza vaccine.

6.1.4. Early Termination Visit

Subjects who withdraw consent will be asked to attend the clinic for safety evaluation as described in Early Termination assessments (Table 2).

Early Termination assessments should be completed within 7 (± 3) days of discontinuation for subjects who discontinue from the study. The following assessments are to be performed:

- Limited physical examination
- Vital signs (blood pressure, heart rate, and body temperature)
- Collect blood for serum chemistry and hematology
- Collect urine for pregnancy test (applicable females only)
- Collect blood sample for immunogenicity assays
- Nasal swab mucosal IgA
- Review eDiary
- Record concomitant therapy/AEs/SAEs

6.1.5. Unscheduled Visits

Unscheduled visits can be planned:

- To obtain additional information to ensure safety to the subject. Additional blood and urine samples may be taken at the discretion of the Investigator.
- To obtain a nasal swab for assessment of potential infection with influenza, SARS-CoV-2 or other respiratory agents (at discretion of PI). If a subject presents within 7 days of IP vaccination with symptoms such as fever, upper respiratory illness, nephritis/cystitis, conjunctivitis or diarrhea, regardless of the severity of the symptoms, an additional nasal swab(s) should be collected and/or analyzed outside of the defined time points. Possible infection with respiratory pathogens (including SARS-CoV-2) may be evaluated by use of a commercial, qualitative and multiplexed polymerase chain reaction (PCR) test for detection of upper respiratory tract pathogens; i.e., the full respiratory panel test in Appendix 3.
- To assess, confirm, and follow-up on out-of-range clinical laboratory test, or vital sign values that will determine a subject's eligibility, or in case of a positive drug screen.
  - The result of the retest will be considered for subject eligibility.

Findings made during unscheduled visits should be reported in the eCRF.

6.2. Study Assessments

6.2.1. Clinical Evaluations

6.2.1.1. Medical History

A complete medical history will include a review of all major body systems. Significant past and present medical history will be obtained by interview during the screening visit.

6.2.1.2. Concomitant Medications

Details of prescription and over-the-counter medications (including vitamins) currently used will be recorded during the screening visit. Concomitant medications will be reviewed at each visit and any new medications will be documented on the eCRF.

6.2.1.3. Demographics

Demographics will be obtained during screening only. Demographics will include the age and race and ethnicity as described by the subject.

6.2.1.4. Physical Examination

6.2.1.4.1. Complete Physical Examination

A complete physical examination will be performed for each subject at the time points specified in the Schedules of Events (Table 2 and Table 3). It will include collection and recording of subject's height and weight. The examination will consist of assessment of the following organs/systems:

- Head
- Eyes
- Ears, nose, throat
- Neck
- Cardiovascular
- Chest
- Respiratory
- Abdomen
- Genitourinary – only if clinically indicated
- Extremities
- Musculoskeletal
- Nervous system – only if clinically indicated
- Dermatological
- Other conditions of note

6.2.1.4.2. Limited Physical Examination

A limited physical exam will be performed for each subject at the time points specified in the Schedules of Events (Table 2 and Table 3). The examination will include the nares (including edema), throat, and lungs (including evaluation for wheezing). A targeted physical examination may be performed on other body systems if indicated based on review of interim medical history or subject symptoms as observed by site or by PI.

6.2.1.5. Vital Signs

Vital signs include oral temperature (°C), blood pressure (mmHg), pulse rate (beats per minute). Vital signs will be obtained at the time points specified in the Schedules of Events (Table 2 and Table 3). Blood pressure will be measured from the subject's arm after the subject has been seated or semi-recumbent for approximately 5 minutes with the arm supported at the level of the heart. Blood pressure will be recorded to the nearest mmHg.

6.2.1.6. Urine Samples

Urine samples for the determination of pregnancy for women of childbearing potential, drug screening, and urinalysis will be collected at the time points specified in the Schedules of Events – IP Administration (Table 2).

A midstream urine sample will be collected for baseline urinalysis by dipstick for specific gravity, pH, glucose, protein, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, and occult blood. Results are obtained by comparing the color block on the canister to those on the reagent strips where the color blocks enclosed by the black box are considered Normal.

If dipstick urinalysis is Abnormal/positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination for white blood cells (WBC), red blood cells (RBC), bacteria, casts, and epithelial cells will be performed. Crystals, casts, and bacteria will only be reported if present.

A urine drug of abuse screen will be performed for detection of opiates, phencyclidine (PCP), cocaine and amphetamines.

6.2.2. Bioanalysis

Blood samples for determination of the concentration of analytes specified in Appendix 3 will be analyzed by a qualified vendor under the responsibility of the Sponsor, using validated analytical methods.

The Investigator must review the laboratory report, document this review, and record any change occurring during the study he considers to be clinically relevant in the AE section in the electronic data capture (EDC) system. Laboratory values outside the normal range will be flagged and their clinical relevance as an AE will be assessed by the Investigator. Laboratory values which are Grade 3 or higher based on the grading scales in Appendix 4 will be reported to the Sponsor within 24 hours of Investigator awareness, and assessment of clinical relevance will be reviewed by the Medical Monitor.

The laboratory analysis will be carried out following the principles of Good Laboratory Practice (GLP) regulations.

6.2.3. Adverse Events

Adverse events will be monitored regularly from informed consent until Visit 05 (28 days after QIV administration). At regular intervals during the study, subjects will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the study will be recorded as well. Solicited and unsolicited signs and symptoms will be reported as AEs after review by the Investigator or designee, either separately using the corresponding Medical Dictionary for Regulatory Activities (MedDRA) terminology of the sign or symptom or combined using the appropriate term. For example, a subject who reported concurrent cough, runny nose, and sneezing assessed by the Investigator as unrelated to vaccine should have those symptoms grouped as a syndromic diagnosis (URI), with the severity based on the most severe AE/symptom. Therefore, as a secondary presentation, subjects with 3 or more concurrent unrelated AEs of cough, runny nose/rhinorrhea, sneezing, sore throat, itchy throat, itchy eyes, congestion, nasal irritation, hoarseness, headache, body aches/myalgia, tiredness/fatigue, malaise, and fever will have those AEs grouped as URI.

For detailed definitions and reporting procedures of AEs, see Section 9.

6.2.4. Electronic Diary

Subjects will be required to complete a reactogenicity eDiary through an application installed on the subject's own personal device. Site will train the subject on the application during Visit 01. All subjects will be asked to monitor and record local reactions, systemic events including temperature, and antipyretic medication usage for 7 days following administration of IP. The reactogenicity eDiary allows recording of these assessments only within a fixed time window, thus providing the accurate representation of the subject's experience at that time. Data on local reactions and systemic events reported in the reactogenicity eDiary will be transferred electronically to a third-party vendor, where they will be available for review by Investigators via an internet-based portal or reports extracted from the portal.

At specified intervals, these data will be retrieved electronically to the Sponsor for analysis and reporting. These data will be reported in the database but do not need to be reported separately by the Investigator in the eCRF as AEs.

Investigators (or designee) will be required to review the reactogenicity eDiary data daily as part of the ongoing safety review. The site will be notified if a subject has missed a diary entry. The eDiary application will remind the subject to populate the diary and will notify the site of the event. If a subject continues to be delinquent the site will contact the subject by phone and will document any missed entries as a protocol deviation. The site will also be notified if a subject has entered a Grade 3 or Grade 4 severity for a symptom. The site must contact the subject to confirm the scoring and determine whether subject should attend the clinic for further follow-up. The Investigator is required to evaluate the symptoms and assess whether they agree with the grading of 3 or higher. Regardless of the PI assessment, the diary entry will not be changed, and the Investigator will verify the score or note a discrepancy in the source.

The Investigator or designee must obtain stop dates from the subject for any ongoing local reactions, systemic events, or use of antipyretic medication on the last day that the reactogenicity eDiary was completed. The stop dates should be documented in the source documents and the information entered in the eCRF.

6.2.4.1. Local Reactions

During the reactogenicity reporting period, subjects will be asked to assess nasal congestion and rhinorrhea after IP administration. All symptoms post-IP will be recorded by the subject in the reactogenicity eDiary. If a local reaction persists beyond the end of the reactogenicity period following vaccine administration, the subject will be requested to report that information. The Investigator will enter this additional information as an AE in the eCRF.

Symptoms emerging after IP treatment will be categorized during analysis as mild, moderate, severe, or potentially life-threatening based on the grading scale in Appendix 2.

If a Grade 3 or Grade 4 local reaction is reported in the reactogenicity eDiary after IP administration, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated.

If a Grade 3 or Grade 4 local reaction (pain, redness, swelling at injection site) is reported after QIV administration at Visit 04 and prior to Visit 05 the site should ascertain whether a site visit is clinically indicated. Grading will be assessed according to FDA Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

Only an Investigator or medically qualified person is able to classify a subject's local reaction to a QIV administration as Grade 4 and must provide a written record of the event. If a participant experiences a confirmed Grade 4 local reaction, the Investigator must immediately notify the Sponsor.

Solicited local reactions following the IP dose include:

- Runny nose
- Stuffy nose/ nasal congestion

6.2.4.2. Systemic Events

During the reactogenicity reporting periods, subjects will be asked to assess headache, fatigue, myalgia, and illness. For the post-IP period, this will include recording the symptoms in the reactogenicity eDiary. The symptoms will be assessed by the subject as none, mild, moderate, severe or medically attended – emergency room visit or hospitalization was required according to the grading scale in Appendix 1.

If a Grade 3 or Grade 4 systemic event is reported in the reactogenicity eDiary after administration of IP, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. If a systemic reaction persists beyond the end of the reactogenicity period following vaccine administration, the subject will be requested to report that information. The Investigator will enter this additional information as an AE in the eCRF. If a Grade 3 systemic reaction is reported after QIV administration (Visit 04) and prior to Visit 05, the site should ascertain whether a site visit is clinically indicated. If a Grade 3 or Grade 4 respiratory event (for example cough or wheezing) has been confirmed by the site, the subject should attend the clinic to provide a nasal swab that is tested as soon as possible for the full panel of respiratory agents.

Only an Investigator or medically qualified person is able to classify a subject's systemic event as Grade 4. If a participant experiences a confirmed Grade 4 systemic event, the Investigator must immediately notify the Sponsor.

Solicited systemic events following the IP dose include:

- Fever
- Chills
- Muscle aches
- Decreased activity
- Decreased appetite
- Headache
- Cough
- Sore throat

6.2.4.3. Fever

In order to record information on fever, a digital thermometer will be provided to subjects with instructions on how to measure oral temperature at home. Temperature will be collected in the reactogenicity eDiary in the evening daily during the reactogenicity eDiary reporting period. It will also be collected at any time during the reactogenicity periods, whether during eDiary data collection (post-IP) or post-QIV interval, when fever is suspected. Fever is defined as an oral temperature of ≥38.0°C (100.4°F). The highest temperature for each day will be recorded in the reactogenicity eDiary. Temperature will be measured and recorded to 1 decimal place and then categorized during PI analysis according to the scale shown in Appendix 1.

If a fever of ≥39.0°C (102.1°F) is reported in the reactogenicity eDiary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Subjects should be instructed that if they record a fever of ≥39.0°C post-administration of QIV, they should contact the site as soon as possible. Only an Investigator or medically qualified person is able to confirm a participant's fever as >40.0°C (>104.0°F). If a participant experiences a confirmed fever >40.0°C (>104.0°F), the Investigator must immediately notify the Sponsor.

6.2.4.4. Antipyretic Medication

The use of antipyretic medication to treat symptoms associated with IP administration will be recorded in the reactogenicity eDiary daily and recorded in the concomitant medications section of the eCRF during the IP reporting period (Day 1 to Day 7).

6.2.5. Immune Response Evaluations

6.2.5.1. Nasal Swabs

Nasal swabs will be collected from subjects to determine mucosal immunity.

Nasal swabs will be collected at the time points specified in the Schedules of Events (Table 2 and Table 3) from all subjects. Individual swabs will be collected from each nostril and placed

in separate vials. Samples will be shipped out for processing then aliquoted for use in various tests.

To evaluate mucosal immunity, an ELISA-based test will be used to measure mucosal IgA antibody titer to HA and total IgA concentration.

6.2.5.2. Blood Collection for Influenza Antibody Testing

Blood samples will be collected and used for serum antibody titers against influenza using MN and research assays. Samples will be collected as indicated in the Schedules of Events (Table 2 and Table 3).

The MN assay will measure all serum antibodies that neutralize the virus and prevent infection of cells and will be analyzed as directed in assay-specific protocols.

Research testing will be performed by the Sponsor or a vendor under supervision of the Sponsor. The research assays include ELISAs or similar methods that may be used to detect anti-influenza immunoglobulin G (IgG) and/or IgA antibodies against influenza HA, HA stalk or NA. Additional assays may be conducted for detection of antibody response to influenza or other respiratory virus, for example NAI assays.

Further details regarding sample collection, processing and shipping can be found in the Laboratory Manual.

6.2.5.3. PBMCs for Cell-mediated Immunity Testing

Blood samples will be collected from all subjects and peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved to be tested for cell-mediated immunity (CMI). Samples are taken as indicated in the Schedules of Events – IP Administration (Table 2).

PBMCs will be used to evaluate T cell responses by ELISPOT assays measuring cytokine secretion upon stimulation with influenza specific peptides. PBMCs may also be evaluated using flow cytometric methods and intracellular cytokine staining to characterize the duration of the responses, and whether memory phenotypes are generated. PBMCs may also be used in a direct ex-vivo ELISPOT assay for evaluation of plasma B cell responses or for detection of memory B cell responses after stimulation.

Further details regarding sample collection, processing and shipping can be found in the Laboratory Manual. PBMC preparation will be the responsibility of each site.

7. STUDY TERMINATION/COMPLETION

7.1. Study Completion and End of Study Definition

A subject will be considered to have completed the study if he or she received the last study vaccination and attended the last protocol-defined follow-up visit.

End of study will be defined as the point when all subjects have completed Visit 05 (28 days after QIV administration). The Sponsor will notify all applicable regulatory agencies in accordance with local requirements when the study has ended.

7.2. Termination of the Study by the Sponsor

This study may be discontinued at any time due to safety concerns, failure to meet expected enrollment goals, administrative reasons, or at the discretion of the Sponsor. Should the study be terminated prematurely, the Sponsor will provide written notification to all Investigators and regulatory authorities and will specify the reason(s) for early termination. The Lead PI must inform the IRB promptly and provide the reason(s) for the termination.

7.3. Halting Rules

Vaccination of subjects with the Study IP will be suspended until after blinded review of safety data by the SRC if any of the following halting rules are met:

1. One or more subjects experience a Grade 4 AE assessed as related to IP.
2. Three or more subjects within an age group experience the same Grade 3 AE assessed as related to IP.
3. One or more subjects experiences an SAE assessed as related to IP.
4. One or more subjects has laryngospasm, bronchospasm, or anaphylaxis associated with the study IP within 72 hours after administration assessed as related to IP.

Any AE or SAE that falls into the above criteria for suspending or terminating study vaccinations must be reported by the Investigator to the Sponsor immediately (within 24 hours).

In the case of AEs meeting the above criteria, the SRC will be convened as quickly as possible, but no later than 3 days after the notification of the event(s). After review of the safety data the SRC will make a recommendation to the Sponsor whether to resume, suspend, or terminate the study. The Sponsor will communicate a decision to suspend or terminate the study to the Investigators. Upon request, the SRC may be provided with an unblinded data set for review.

Subsequent review of serious, unexpected, and related AEs by the Medical Monitor, SRC, IRB, the Sponsor(s), or the Regulatory Agency may also result in suspension of further trial interventions/administration of study product at a site. The FDA and study Sponsor(s) retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

7.4. Removal of Subjects from the Study

Subjects have the right to withdraw from the study at any time for any reason, including personal reasons. A subject can withdraw without giving a reason. The Investigator should however try to determine why a subject withdraws from the study and document the reason for withdrawal in the source documents and in the EDC system.

Subjects may be withdrawn from the study in the event of:

- Meeting any of the halting rules in Section 7.3
- A severe AE or SAE;
- Difficulties in obtaining blood or other samples;
- Failure of the subject to comply with the protocol requirements or to cooperate with the Investigator.

Subjects must be withdrawn from the study in the event of:

- Withdrawal of consent
- Lost to follow-up
- Death
- Termination of the study by the Sponsor (described in Section 7.2)
- For safety reasons, it being in the best interest of the subject that he/she be withdrawn, in the Investigator's opinion;
- A positive pregnancy test (the subject or, if reported by a male subject, his female partner), or if the subject/partner is non-compliant with the contraception requirements (see Section 4.1);
- Lab confirmed infection with SARS-CoV-2
- Development of a medical condition that requires concomitant treatment with a prohibited therapy (see Section 5.9.2);
- Breaking of the randomization code during administration of the study drug. If the code is broken for safety reporting purposes, the subject may remain in the study; however, in such cases subject and site staff will remain blinded to IP treatment.

The monitor and Sponsor will be informed in the event of a subject being withdrawn from the study. In case of withdrawal due to an SAE (for details on AE reporting see Section 9.5), the Sponsor should be notified within 24 hours; in case of withdrawal for other reasons; the Sponsor should be notified within 2 days from the event.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (AE, withdrawal of consent, etc.) should be contacted by phone for a safety follow-up 4 weeks after vaccination. In case of an AE, the appropriate follow-up will be done.

Subjects who are withdrawn from the study will not be replaced.

8. STATISTICAL METHODS

The Statistical Analysis Plan (SAP), including table shells will be finalized prior to database lock. The final analysis will be completed after the last subject has had Visit 05 (28 days following the QIV dose) and the data base has been locked.

Analyses will be done using SAS software version 9.4 or higher.

Early reviews may be conducted on an expedited basis and included in an interim report, to aid in decision-making for future studies.

- Early reviews prior to database lock will be done on "snapshots" of the data available at the time of analysis. These snapshots will be stored as a permanent record of the trial.
- These reviews will not affect the conduct of this trial.
- Results will be presented by group; reports will display no subject level data.
- Personnel conducting immune response assays and conducting procedures or assessments of subjects will not have access to any data that would unblind them to individual subject treatment codes.

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

8.1. Analysis Datasets

The safety set will include any subject who receives administration of IP.

The full analysis set (FAS) will include all subjects randomized, received IP and had at least one post baseline assessment.

A per protocol set (PPS) may also be evaluated, which will include all subjects in the FAS with no major protocol violations or deviations.

8.2. Subject Disposition and Baseline Characteristics

8.2.1. Sample size

The sample size of 15 subjects per cohorts 1 and 2, and 90 subjects per cohort 3 were chosen based on reports of studies of other vaccines in early-stage development. This study is not powered for any formal comparisons. See Section 1.4 for probability of detecting an AE.
Subject Characteristics Data Set

Demographics will include by-treatment summaries of age, height, weight, body mass index (BMI), race, ethnicity, and gender.

Other baseline characteristics may be summarized.

8.3. Safety Analyses

Safety and tolerability of the Sing2016 M2SR influenza vaccine alone and when followed by a licensed vaccine as assessed by Investigator review of subject eDiaries, solicited and unsolicited AEs, treatment-related AEs, SAEs, vital signs, and physical examinations.

AEs will be collected for a period of 28 days following the IP and the QIV doses. However, solicited AEs will be collected by eDiary only after IP administration and not after the licensed vaccine administration. It is therefore anticipated that the summary of AEs post-IP and post-QIV will be different.

8.3.1. Exposure

Vaccine exposure will be summarized by actual dose, percent of expected dose, and percent of subjects receiving the expected dose.

8.3.2. Adverse Events

Treatment-emergent AEs (local and systemic) will be summarized as N (%) with AEs, with related-AEs, and by worst severity.

Incidence of treatment-emergent adverse events (TEAEs) will be reported by MedDRA-coded system organ class (SOC), preferred term (PT), and maximum severity.

Incidence of most frequent TEAEs and most frequent treatment-related TEAEs will be reported by SOC and PT

Listings of subjects who died, discontinued the study due to an AE, or experienced an SAE will be provided.

Dose-site reactions will be presented by reaction and severity.

Post-administration symptoms (eDiary) will be summarized by time point, worst occurrence, and days with symptoms.

8.3.3. Vital Signs

Vital sign results and change from baseline results will be summarized by timepoint using descriptive statistics.

8.3.4. Physical Examination

Abnormal findings of clinical significance in the physical examination will be listed.

8.4. Immunogenicity

Serum antibody, mucosal antibody response will be summarized using descriptive statistics by sampling timepoint for all subjects and by baseline values.

Seroconversion rates will be presented by timepoint and by baseline starting values.

8.5. Appropriateness of Measurements

The assessments which will be made in this study are standard, and are generally recognized as reliable, accurate, and relevant.

9. ADVERSE EVENT REPORTING

9.1. Definitions

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, and whether related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal result of diagnostic procedures, including clinical laboratory test abnormalities.

AEs will be monitored from the time of consent until Visit 03 (Day 28 after the IP dose administration) and from Visit 04 until Visit 05 (Day 28 after the QIV dose administration). SAEs or medically attended events will be documented from the time of consent until Visit 05.

Serious Adverse Event

An SAE is any untoward medical occurrence that meets any of the following conditions:

- results in death;
- is life-threatening, i.e., the subject was at risk of death at the time of the event (e.g., ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing inpatient hospitalization:
    - Hospitalization refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalization for an elective procedure, or routinely scheduled treatment for a pre-existing condition that has not worsened, is not an SAE.
- results in persistent or significant disability/incapacity, i.e., causing substantial disruption of the subject's ability to conduct normal life;
- is a congenital anomaly/birth defect;
- is medically significant, i.e., may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject's health or may require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

Unlisted (Unexpected) Adverse Event

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information (FluGen IB).

Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an unexpected serious adverse reaction to the IP.

The Sponsor is to determine if the event is unexpected or not and if unexpected and to report the SUSAR to the Lead PI and FDA. The Lead PI will report any SUSAR(s) to the central IRB. For death and life-threatening cases, reporting should be done within 7 days and follow-up information with details provided within an additional 8 days. All other SUSARs have to be reported within 15 days.

9.2. Intensity of Adverse Events

All AEs will be assessed by the clinician using the grading system from FDA vaccine toxicity criteria (Appendix 2 for vital signs and systemic AE assessment; Appendix 4 for laboratory AE assessment). For events not included in the protocol-defined grading system, then the following guidelines will be used to quantify intensity.

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

Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Life threatening: any adverse drug experience that places the subject or subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

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

9.3. Causality Assessment

The clinician's assessment of an AE's relationship to test article (vaccine or placebo) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.

Not Related – The AE is not related to the study product if there is evidence that clearly indicates an alternative explanation. If the subject has not received the study product, the timing of the exposure to the study product and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that strongly suggest an alternative explanation, then the AE is not related.

9.4. Outcome

The outcome of each AE must be rated as follows:

- Recovered/resolved;
- Recovering/resolving;
- Not recovered/not resolved;
- Recovered with sequelae/resolved with sequelae;
- Fatal;
- Unknown.

9.5. Recording of Adverse Events

All (S)AEs occurring during the clinical investigation must be documented in the source documents and eCRF.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study drugs in the EDC System. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study (including the in-person follow-up period) will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilization or until final database lock. If necessary, to obtain additional information to ensure safety of the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases, follow-up will be the responsibility of the treating physician.

9.6. Reporting of Serious Adverse Events to the Sponsor for Pharmacovigilance

All SAEs, independent of the circumstances or suspected cause, must be recorded on a Serious Adverse Event Form by the Investigator and reported to the Sponsor within 24 hours of knowledge of the event. The SAE may be initially reported by sending the completed SAE Form to FluGen by email (Safety@flugen.com) within 24 hours of knowledge of the event.

A copy of the Medwatch or Council for International Organizations of Medical Sciences (CIOMS) initial report and follow-up reports will also be submitted by the Sponsor to the Division of Microbiology and Infectious Diseases (DMID) Pharmacovigilance Group:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, US
SAE Hot Line: 1-800-537-9979 (US)
SAE FAX Phone Number: 1-800-275-7619
SAE Email Address: PVG@dmidcroms.com

Upon notification, the Medical Monitor will evaluate the SAE report(s) within 1 working day of receipt to determine the regulatory reporting priority or, if necessary, request additional information from the site to make an evaluation.

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the SAE Form matches the information recorded in the source documents and in the EDC system for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be submitted to FluGen and IRB (as applicable) until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

9.7. Pregnancy

All initial reports of pregnancy in subjects or in partners of male subjects must be reported to the Sponsor by the Investigator within 24 hours of his/her knowledge of the event. This is done by contacting FluGen via email (Safety@flugen.com). Any subject who becomes pregnant during the study must be promptly withdrawn from the study (Code of Federal Regulations [CFR] Section 8).

The Investigator will contact the subject at the expected time of delivery for follow-up and will provide the pregnancy outcome to the Sponsor. Abnormal pregnancy outcomes (e.g., spontaneous or induced abortion, stillbirth, neonatal death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.

9.8. Reporting of Serious Adverse Events to Competent Authorities/Institutional Review Boards

Adverse events reporting, including SUSARs, will be carried out in accordance with applicable local regulations.

All SUSARs will be the subject of expedited reporting. The Sponsor and Investigator shall ensure that all relevant information about a SUSAR that is fatal or life-threatening is reported to the relevant competent authorities and the site IRB within 7 days after knowledge by the Sponsor of such a case, and that relevant follow-up information is communicated within an additional 8 days, or according to the IRB's required time frame. All other SUSARs will be reported to the relevant competent authorities and IRB within 15 days after knowledge by the Sponsor of such a case. FluGen will provide Investigators with all details of all SAEs reported to regulatory authorities.

9.9. Medical Monitor

The Medical Monitor is an independent clinician (i.e., not directly employed by the Sponsor or the site) responsible for safety oversight of the study. He/she should have no apparent conflict of interest and cannot be under the supervision of the PI or other Investigators or research staff. The Medical Monitor will oversee interventions, interactions, data matching, data collection and data analysis, review the clinical monitoring plan, review SAE and SUSAR reports (Section 9.6), review AEs which may prompt a study halt (Section 7.3), participate in the SRC (Section 9.10), participate in decisions regarding emergency unblinding (Section 5.6), participate in review of Grade 3 and Grade 4 laboratory abnormalities, and review summary AE data prior to final database lock. The Medical Monitor is responsible to promptly report his/her observations to the IRB or other designated official. The Medical Monitor has the authority to stop the study, remove individual human subjects from a research protocol if needed and take any steps necessary to protect the safety and well-being of the subjects until the IRB can assess the monitor's report. The Medical Monitor will also participate via phone in site training and will be available to answer questions related to study eligibility and safety for the duration of the study and may discuss the research protocol with Investigators.

9.10. Safety Review Committee

Formal safety oversight will be under the direction of an SRC, which is composed of an independent group of experts including two clinicians and a statistician who will be available to review blinded safety data, as needed. The SRC will monitor subject safety and advise the Sponsor. SRC members will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflict of interest related to the study.

The SRC will operate under the rules and procedures of a Sponsor-approved charter.
In addition, there will be 2 scheduled SRC meetings for informational safety updates. The first meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 1. The second meeting of the SRC will consist of the cumulative safety data available through 7-days post-vaccination of Cohort 2. The safety reports generated for the SRC meeting are to be subjected to at least one round of review towards establishing a cleaned and QCed dataset. The purpose of these scheduled meetings is informational and not for gating decisions that determine progress to each successive cohort.

For informational safety reviews and if study halting rules are met, the SRC will be provided applicable data including, but not limited to, enrollment, demographic, dosing, laboratory and

safety data, as defined in the protocol and the SRC charter. The SRC may receive data in aggregate and presented by treatment group, but without the treatment group identified. The SRC may review aggregate safety data for increased rate of occurrence of serious suspected adverse reactions. The SRC may be unblinded to study treatment, as needed, to assess safety issues. As an outcome of each review/meeting, the SRC will advise the Sponsor of its findings and make recommendations with respect to continuation of the study.

Throughout the study, the SRC will be convened if halting rules are met. The SRC also may be convened at the request of the Investigator, Medical Monitor, Sponsor or any SRC member, if they have cause for concern regarding subject safety. The SRC can recommend study enrollment and vaccinations be stopped if AEs that meet the halting criteria are reported or for any overriding safety concern. Further details regarding composition and operation of the SRC are provided in the SRC Charter.

10. ETHICAL ASPECTS

10.1. Study-Specific Design Considerations

Potential subjects will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and who provide their consent voluntarily will be enrolled in the study.

10.2. Regulatory Ethics Compliance

10.2.1. Investigator Responsibilities

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirements, and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB, or the regulatory authorities.

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

10.2.2. Institutional Review Board

An IRB should safeguard the rights, safety, and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Sponsor (or Investigator where required) will provide the IRB with current and complete copies of the following documents:

- final protocol and, if applicable, amendments;
- Sponsor-approved ICF (and any updates or any other written materials to be provided to the subjects);
- Subject recruiting materials;
- IB (or equivalent information) and addenda;
- available safety information;

- information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IRB);
- Clinical trial agreement;
- any other documents that the IRB may require to fulfill its obligation.

This study will be undertaken only after the IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IRB and the documents being approved.

During the study, the Sponsor (or Investigator where required) will send the following documents and updates to the IRB for its review and approval, where appropriate:

- protocol amendments;
- revision(s) to the ICF and any other written materials to be provided to the subjects;
- new or revised subject recruiting materials approved by the Sponsor;
- revisions to compensation for study-related injuries or payment to subjects for participation in the study;
- IB addenda or new edition(s);
- summaries of the status of the study at intervals stipulated in guidelines of the IRB (at least annually);
- reports of AEs that are serious, unlisted, and associated with the IP;
- new information that may adversely affect the safety of the subjects or the conduct of the study;
- deviations from or changes to the protocol to eliminate immediate hazards to the subjects;
- report of death of any subjects under the Investigator's care;
- notification if a new Investigator is responsible for the study at the clinical site;
- any other requirements of the IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IRB as soon as possible.

The Sponsor (or Investigator will notify the IRB about the study completion within 90 days after the end of the study (defined as last subject last visit [LPLV]).

10.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the Investigator or an authorized member of the clinical staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may refuse to participate or withdraw consent to participate at any time, without penalty or loss of benefits to which the subject was entitled. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities, National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) representative as part of its human subject protection oversight activities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subject. The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject.

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety, quality, and utility of the IP used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

11. ADMINISTRATIVE REQUIREMENTS

11.1. Protocol Amendments

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IRB approval nor when the relevant regulatory authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IRB must be provided to the Sponsor or his designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB only needs to be notified.

11.2. Subject Identification, Enrollment, and Screening Logs

The Investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

The Investigator must also complete a subject screening log which reports on all subjects who were seen to determine eligibility for inclusion in the study.

11.3. Source Documentation

The Investigator will maintain a list of qualified staff to whom study responsibilities have been delegated. These individuals authorized to fulfill these responsibilities should be outlined and included in the Delegation of Authority Form.

Source documents are original documents, data, and records for which the study data are collected and verified. Example of such source documents may include, but are not limited to, subject hospital/medical records, laboratory reports, physicians' and nurses' notes, subject eDiaries and correspondence. At a minimum, source documentation must be available for the following: subject identification, eligibility, and study identification; date of subject consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, drug receipt/dispensing/return records, study drug administration information, laboratory printouts (if not available digitally), date of study completion, and reason for early discontinuation of study drugs or withdrawal from the study, if applicable.

The Investigator and study staff are responsible for maintaining a comprehensive filing system of all subject records that are readily available to support monitoring activities in compliance with ICH-GCP guidelines and regulatory and institutional requirements for the protection of confidentiality of subjects. The Investigator shall supply the Sponsor or designee, on request,

with any required background data from the study documentation or clinic records. Such requests may occur, for example, when documents are illegible or when errors in data transcription are suspected. In case of requests for audit inspections and/or queries from National Authorities/Regulatory Agency, it will be necessary to have access to the complete study records, provided that subject confidentiality is maintained.

No study documents should be discarded without prior written agreement between the Sponsor and the Investigator. Should storage no longer be available to archive source documents or must be moved to an alternative location, the site should notify the key sponsor contact prior to the shipping of documents.

11.4. Case Report Form Completion

Authorized study site personnel will complete eCRFs designed specifically for this study and completion guidelines will be provided. An eCRF is required and must be completed for each subject enrolled into the study and will be available for all data required to be entered into the clinical database and must match the data contained in the study specific source documentation. The Investigator will ensure that the eCRFs are accurate, complete and legible. The Investigator will ensure that source documents that are required to verify the validity and completeness of data transcribed on the eCRFs are never obliterated or destroyed. As required by the protocol, eCRFs should also be completed for those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the eCRF and thorough efforts should be made to clearly document outcome.

The eCRFs will be maintained in an electronic data collection system for this study. After the Investigator or designees have been appropriately trained, they will be given access to the EDC system and will be able to enter the data required by the protocol. Any change of data will be made via the EDC system, with all changes tracked by the system to provide an audit trail.

11.5. Study Monitoring

The Sponsor is responsible for assuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the eCRFs. Subject confidentiality will be maintained.

In addition, clinical monitors designated by the Sponsor will periodically contact the site, primarily to conduct remote monitoring visits but with option for on-site visits if deemed necessary. The remote and on-site monitoring visits will be conducted as frequently as necessary to ensure that all aspects of the study are carefully monitored for compliance with applicable government regulations with respect to current GCP practice and the current SOPs. The visits will be conducted in accordance with the Sponsor's SOP and Clinical Monitoring Plan.

The unblinded clinical monitor will be limited to observation (via review of documentation or if deemed necessary in real-time whether remote or on-site) of IP preparation, delivery device filling, adherence to randomization to treatment group, and storage of IP and IP accountability. They will not participate in activities related to subject assessments.

In general, the Investigator agrees to fully cooperate with the monitor, allow the monitor direct access to all relevant documents, to allocate his time and the time of his staff to the monitors to discuss any findings and any relevant issues as needed.

11.6. Data Management

Electronic data collection will be used to enter study data. During the data collection process, automated quality assurance programs will be used to identify missing data, incorrect data and other data discrepancies. Requests for data clarification or correction will be queried to the investigative study site for resolution via the EDC system.

Quality assurance and quality control systems will be implemented and maintained to ensure that the data are generated, recorded and reported in compliance with the protocol, GCP, and applicable regulatory requirements. Data collection and storage will provide an audit trail, security mechanisms and electronic signature capabilities that meet the requirements of FDA Title 21 of CFR Part 11 regarding electronic records and electronic signatures.

Data security will be controlled through appropriate and specific restriction of access to only data and systems required by individual users to accomplish their roles in the data management process. Individual login and password protections will be employed. The database will exist on physically secured servers. Data backups will be done regularly and will be stored in a separate facility.

11.7. Data Quality Assurance

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designee.

Written instructions will be provided for the collection, preparation, and shipment of biological test samples.

The Sponsor or designee will review the paper source and eCRF entries for accuracy and completeness during remote monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy will be verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

11.8. On-Site Audits

Representatives of the Sponsor's clinical quality assurance department may visit the clinical site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eCRF system. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or his designee.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

11.9. Study Termination

The Sponsor has the right to terminate the study at any time. In case of an early termination of the study for safety reasons, or temporary halt by the Sponsor, the IRB should be notified within 15 calendar days and should be provided with a detailed written explanation for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

11.10. Record Retention

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all paper source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

11.11. Use of Information and Publication

All information, including but not limited to, information regarding the Sing2016 M2SR vaccine or the Sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this information only to accomplish this study, and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the study drug, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report (CSR) generated under the responsibility of the Sponsor and will contain EDC system data from all clinical sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating Investigator.

Clinical narratives may be written for the following events (for example):

- All deaths (irrespective of drug relationship)
- All other SAEs during treatment with the study drugs
- All discontinuations of the study drugs due to AEs (irrespective of drug relationship)
- Any events of special interest explicitly requested by the regulatory agencies
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment group).

The Sponsor and Medical Monitor will sign off the final version of the CSR. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (LPLV).

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, the Investigator must first request to do so in writing to the Sponsor at least 60 days before submission or presentation of the study data. A copy of the manuscript or presentation must also be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials to the best of the Sponsor's ability and only if Sponsor agrees to allow Investigator to submit such

materials to an outside party. The Investigator will withhold such publication for up to an additional 60 days or until the Sponsor provides its express written consent for Investigator to move forward with publication or presentation of any study data. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content but in no case, will Investigator have the right to publish, present or share with any outside party study data and/or information without the express written consent of the Sponsor. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

11.12. Results

The Sponsor will register the existence of a clinical study and disclose its results as required by law.

11.13. Investigator Indemnity

The Sponsor holds and will maintain an adequate insurance policy covering damages arising out of Sponsor-sponsored clinical research studies.

The Sponsor will indemnify the Investigator and hold him/her harmless for claims related to damages arising from the investigation, provided that the study drugs were administered under the Sponsor's or deputy's supervision and in strict accordance with accepted medical practice and the study protocol.

The Investigator must notify the Sponsor immediately upon notice of any claims or lawsuits.

11.14. Confidentiality

Subject confidentiality is strictly held by the participating Investigators, their staff, and the Sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

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

The clinical monitors or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The Investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. Throughout this study, all data will be linked to the eCRF via a unique identification number. The data will be blinded correspondingly in all data analyses. The Investigator should keep a subject enrollment log showing codes, names and

addresses. The Investigator should maintain documents that are not for submission to the Sponsor (such as written consent forms) in strict confidence.

However, in compliance with the ICH Guidelines and in fulfillment of its obligations to FluGen to verify compliance with this protocol, FluGen Inc. or its designee requires that the Investigator permit FluGen designated monitors, representatives from any Regulatory Authority, FluGen designated auditors, or the appropriate Independent Ethics Committee, to review the subject's primary medical records (source data or documents) including, but not limited to, laboratory test result reports, admission and discharge summaries, and SAEs occurring during the study. Should access to such medical records require a waiver or authorization separate from the statement of informed consent, the Investigator will obtain such permission in writing from the subject before the subject is entered into the study.

12. REFERENCES

Ambrose CS, Wua X, Jones T, Mallory RM. The role of nasal IgA in children vaccinated with live attenuated influenza vaccine. Vaccine 2012; 30:6794–801.

Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. J Infect Dis. 2000;181(3):1133-7.

CDC. National Center for Immunization and Respiratory Diseases (NCIRD). Disease Burden of Influenza. 05 October 2020. Available at: https://www.cdc.gov/flu/about/burden/index.html Accessed on: 17 February 2021.

Clements ML, Betts RF, Tierney EL, et al. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J Clin Microbiol. 1986;24(1):157-60.

Eick AA, Wang Z, Hughes H, et al. Comparison of the trivalent live attenuated vs. inactivated influenza vaccines among U.S. military service members. Vaccine. 2009;27:3568-75.

Grohskopf LA, Shay DK, Shimabukuro TT, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2013–2014. MMWR Recomm Rep. 2013;62(RR07):1-43.

Grohskopf LA, Leslie Z Sokolow, Sonja J Olsen. Prevention and control of influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices – United States, 2015–16 Influenza Season. MMWR Recomm Rep. 2015;64(30):818-25.

Grohskopf LA, Alyanak E, Broder KR, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2019–20 Influenza Season. MMWR Recomm Rep. 2019;68(3):1–21.

Jin H and Subbarao K. Live attenuated influenza vaccine. In: Oldstone MBA, Compans RW, eds. Influenza pathogenesis and control, Vol II. Cham, Switzerland: Springer International Publishing. 2015:181–204.

Lanthier PA, Huston GE, Moquin A, et al. Live attenuated influenza vaccine (LAIV) impacts innate and adaptive immune responses. Vaccine. 2011;29:7849–56.

Monto AS, Ohmit SE, Petrie JG, et al. Comparative Efficacy of Inactivated and Live Attenuated Influenza Vaccines. N Eng J Med. 2009; 361:1260-7.

Ohmit SE, Victor JC, Rotthoff JR, et al. Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. N Engl J Med. 2006;355:2513-22.

Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12: 36–44.

Tamura S, Tanimoto T, Kurata T. Mechanisms of broad cross-protection provided by influenza virus infection and their application to vaccines. Jpn J Infect Dis. 2005;58:195–207.

Treanor JJ, Kotloff K, Betts RF, et al. Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following

challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. Vaccine. 1999;18(9-10):899-906.

Treanor JJ, Talbot HK, Ohmit SE, et al. Effectiveness of Seasonal Influenza Vaccines in the United States During a Season with Circulation of All Three Vaccine Strains. Clin Infect Dis. 2012;55:951–9.

Wang Z, Tobler S, Roayael J, et al. Live attenuated or inactivated influenza vaccines and medical encounters for respiratory illnesses among US military personnel. JAMA. 2009; 301:945-53.

Wilkinson TM, Li CK, Chui CS, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med. 2012;18(2):274-80.

Zhu W, Higgs BW, Morehouse C, et al. A whole genome transcriptional analysis of the early immune response induced by live attenuated and inactivated influenza vaccines in young children. Vaccine. 2010;28:2865–76.

13. APPENDICES

APPENDIX 1: EDIARY FOR IP DOSING PERIOD

SYMPTOM	INTENSITY LEVEL	DESCRIPTION
1. Temperature	###.#	Degrees Celsius or Fahrenheit to one decimal point
2. Chills 3. Muscle Aches 4. Decreased Activity 5. Decreased Appetite	0	None
	1	Mild - No interference with activity
	2	Moderate - Some interference with activity
	3	Severe - Significant interference, prevents daily activity
	4	Medically attended – emergency room visit or hospitalization was required
6. Headache	0	None
	1	Mild - No interference with daily activity
	2	Moderate - Some interference with daily activity
	3	Severe - Significant interference, prevents daily activity
	4	Medically attended – emergency room visit or hospitalization was required
7. Cough	0	None
	1	Mild - Noticeable but does not interfere with daily activity or sleeping
	2	Moderate - Moderate discomfort/interferes with daily activity or sleeping
	3	Severe - Significant discomfort/prevents daily activity
	4	Medically attended – emergency room visit or hospitalization was required
8. Sore Throat	0	None
	1	Mild - Noticeable but does not interfere with eating and/or drinking
	2	Moderate - Moderate discomfort. Interferes with eating and/or drinking
	3	Severe - Significant discomfort/prevents eating and/or drinking
	4	Medically attended – emergency room visit or hospitalization was required
9. Stuffy Nose/Nasal Congestion	0	None
	1	Mild - Noticeable but does not interfere with daily activity
	2	Moderate - Moderate discomfort/interferes with breathing from nose
	3	Severe - Not being able to breath from nose, or prevents daily activity
	4	Medically attended – emergency room visit or hospitalization was required
10. Runny Nose	0	None
	1	Mild - Noticeable but does not interfere with daily activity
	2	Moderate - Moderate discomfort/interferes with daily activity

	3	Severe - Significant discomfort/prevents daily activity
	4	Medically attended – emergency room visit or hospitalization was required

APPENDIX 2: TOXICITY GRADING SCALE FOR HEALTHY VOLUNTEERS ENROLLED IN PREVENTATIVE VACCINE CLINICAL TRIALS

Local Reactions

As a component of limited physical exam by Investigator and during the solicited reporting periods, reactogenicity events will be assessed with results reported in the eDiary post-IP administration. If a Grade 3 or 4 local reaction is reported in the eDiary then the site Investigator must contact the subject within 18 hours to discuss the symptom in detail and confirm the scoring. Following an eDiary report of a Grade 3 or 4 local reaction, the site will determine whether additional action such as a site visit is warranted. If a Grade 4 local reaction is reported then the Investigator or trained medical professional must confirm the score and must immediately notify the Sponsor.

Please score reactions as follows:

Local reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Stuffy nose / Nasal congestion	Does not interfere with activity	Use of saline or other decongestant > 24 hours or interferes with activity	Repeated use of decongestants or significantly interferes with daily activity	Emergency room (ER) visit or hospitalization
Runny nose / Nasal rhinorrhea	Does not interfere with activity	Use of saline or other decongestant > 24 hours or interferes with activity	Significantly interferes with daily activity	Emergency room (ER) visit or hospitalization

Vital signs

Please score items based on the following guidance (from FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2008) and note whether clinically significant:

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.

** Oral temperature; no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic Symptoms

Please score items based on the following guidance (from FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2008):

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

APPENDIX 3: LABORATORY ASSESSMENTS

• Urine drug screen	Opiates, PCP, Cocaine and Amphetamines
• Serology:	HIV, HCV, HbsAg
• Urinalysis:	Dipstick: specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase In case of positive dipstick results for protein, blood, nitrite and/or leukocyte esterase, the sediment will be examined microscopically for red blood cells, white blood cells, bacteria, casts, and epithelial cells. Crystals, casts, and bacteria will only be reported if they are present.
• Hematology:	Hemoglobin, white blood cells with differential (including neutrophils, lymphocytes, and eosinophils), platelets
• Biochemistry:	Albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate (or carbon dioxide), calcium, chloride, potassium, sodium, total bilirubin, direct bilirubin[a], total protein, creatinine, C-reactive protein, blood urea nitrogen (or urea)
• Abbreviated respiratory panel:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Respiratory Syncytial Virus, Influenza A, and Influenza B
• Full respiratory pathogen panel:	To include: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A (including subtypes H1, H3), Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, and Respiratory Syncytial Virus. May include: Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae, and Mycoplasma pneumoniae and Influenza subtype H1-2009.
• At screening and prior to IP dose administration on Day 1, a urine pregnancy test will be performed for women of childbearing potential	
• At screening, prior to IP dose administration on Day 1, and on Day 8, a nasal swab will be collected. A PCR test for SAR-CoV-2 will be performed on the screening and Day 8 samples to assess eligibility. The Day 1 (V01) sample will be tested for an abbreviated set of respiratory agents. The Day 1 (V01) and Day 8 (V02) samples will be collected, combined in a shipment for the central lab and batch tested. The Day 1 (V01) sample will further be tested for the full respiratory pathogen panel using a multiplexed PCR assay if subject presents within 7 days of IP vaccination with symptoms of fever, upper respiratory illness, nephritis/cystitis, conjunctivitis or diarrhea.	

a. To be assessed if total bilirubin is elevated above normal range.

APPENDIX 4: LABORATORY ADVERSE EVENTS

Please score items based on the following guidance (from FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials):

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.4	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--

Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in liver function test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when liver function test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***"ULN" is the upper limit of the normal range.

Grading Scale for Urinalysis AEs

Please score items based on the following guidance (modified from FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials):

Urine *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Protein	1+	2/3+	Requires medical attention	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon kit defined parameters. Kit defined reference ranges should be provided to demonstrate that they are appropriate.