A Phase 1b, double-blind, randomized, dose-escalating, age de-escalating, placebo-controlled study to assess the safety and immunogenicity of one or two doses of Sing2016 M2SR H3N2 influenza vaccine delivered intranasally in a healthy pediatric population 6 months through 17 years of age.

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IND Sponsor: DMID, NIAID

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Version Number: 5.0

Date: 6 Dec 2023

STATEMENT OF COMPLIANCE

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

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

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), and/or 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice, and the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and DMID
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

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

Site Investigator Signature:

Signed: _____ Date: _____

Name
Title

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1. PROTOCOL SUMMARY

1.1 Synopsis

Rationale for Proposed Clinical Study:
The influenza virus circulates widely in the United States with peaks usually between December and February of each year. Young children (≤ 2 years), older adults (≥ 65 years), and persons with underlying medical problems are especially vulnerable to more severe disease and may suffer dire consequences, including hospitalization and even death. Prior to widespread vaccinations, influenza globally claimed 40 -70 million lives annually due to influenza-related complications [1]. Today, in the United States alone, despite wider spread vaccination efforts, influenza continues to claim 30,000 to 49,000 lives annually with approximately 200,000 hospitalizations. Although vaccines are available and recommended for nearly the entire US population, not all people receive annual influenza vaccination and, according to the US Flu-VE Network, the effectiveness of our currently licensed vaccines is typically only 40-60% [2]. The overall effectiveness of currently licensed influenza vaccines against H3N2 influenza strains during influenza seasons with a mismatch between the vaccine and the circulating strain can be as low as 0% with an average of only 23% (95% Confidence Interval [CI] 2% to 40%) [3]. Improved vaccines against influenza are needed. FluGen, Inc. has developed a novel influenza vaccine which is designed to provide broader-spectrum immunity against multiple influenza type A viruses, especially in pediatric and geriatric patients.

The FluGen vaccine that is being evaluated in this study is a live vaccine product called Sing2016 M2SR H3N2. It has the ability to infect respiratory epithelial cells in a manner similar to wild-type influenza virus and to induce an immune response against a broad spectrum of type A influenza viral strains [4]. Given that Sing2016 M2SR H3N2 has a defective M2 gene, unlike wild-type viruses, it is unable to replicate and generate progeny in animals and in humans, making its infectivity self-limiting. This attenuation is expected to improve safety while maintaining the ability to elicit an immune response. Furthermore, like FluMist®, the US-licensed live attenuated intranasal influenza vaccine, the Sing2016 M2SR H3N2 vaccine induces both a mucosal and cell-mediated immune response and is administered intranasally. However, unlike FluMist®, the Sing2016 M2SR H3N2 vaccine has been demonstrated to induce immune responses in animal models even in the presence of pre-existing antibodies.[5] Furthermore, Sing2016 M2SR H3N2 induces antibodies in animal models against the highly conserved stem region of hemagglutinin (HA) as well as against neuraminidase (NA) surface proteins on the surface of the influenza virus.[6] These features may improve its ability to induce immune responses against a broader spectrum of influenza type A strains and potentially may prolong immunity despite on-going drift mutations in the influenza type A virus.[4] Early stage clinical trials with the M2SR vaccine platform vaccines have included 3 studies conducted in the US and an influenza challenge study conducted in Belgium. Please refer to the Investigator's Brochure (IB) for more information about these trials. The 3 US studies included two dose-ranging studies in adults and one study in pediatric participants aged 9 to 17 years. The DMID 17-0012 study, entitled "A Phase I Trial to Evaluate the Safety and Immunogenicity of an Influenza Vaccination Strategy Including a H3N2 M2SR Prime followed by a Seasonal Quadrivalent Inactivated Vaccine Boost in a Pediatric Population 9-17 years old" (BB-IND 18170; sponsored by DMID, NIAID), enrolled 43 males and non-pregnant females ages 9 to 17 years old, inclusive.

Participants were randomly allocated in a 1:1 ratio to receive one 10^8 $TCID_{50}$ dose of Bris10 M2SR vaccine or placebo administered intranasally (IN) followed by one dose of licensed quadrivalent influenza vaccine (IIV4) administered intramuscularly 3 months later. Enrollment was completed on September 20, 2019 with 43 participants enrolled. Twenty-eight participants were in the 9–14-year-old age range and 15 participants in the 15–17-year-old age range. There were no reported serious adverse events (SAEs), adverse events (AEs) of special interest, or halting rules met. Preliminary results for this study are posted on clinicaltrials.gov, study identifier NCT03553940.

The M2SR vaccine platform vaccines have been found to be safe and well-tolerated during these 4 studies, including administration of doses as high as 10^9 $TCID_{50}$ in the second adult dose ranging study.[4] In adults, M2SR platform vaccines induce T-cell responses as well as serum and mucosal antibody responses to both matched and drifted influenza strains. Protection against a highly drifted H3N2 influenza challenge strain was observed in a subset of volunteers who received a single intranasal dose of 10^8 $TCID_{50}$ of monovalent H3N2 M2SR one month prior to challenge.

In this study, we propose to perform a Phase 1b, double-blind, dose-escalating, age de-escalating study of the Sing2016 M2SR H3N2 vaccine in children ages 6 months to 17 years. The purpose of the study is to assess the safety, tolerability/reactogenicity, and immunogenicity of the vaccine.

Study Design:
This is a Phase 1b, randomized, double-blind, dose-escalating, age de-escalating, placebo-controlled study of 200 children, ages 6 months to 17 years. To allow for simultaneous enrollment at the four research sites, the number of children in each cohort and the total number of children in the entire study may slightly deviate from the targeted numbers mentioned throughout the protocol. The study will enroll seven cohorts of children. The study design includes pre-planned Safety Review Committee (SRC) reviews.

The first two groups to be vaccinated will be Cohorts 1 and 2. Cohort 1 consists of 45 children 9-17 years old. Thirty of them will receive one dose of the vaccine at a dose of 10^9 $TCID_{50}$, and 15 will receive one dose of placebo. Cohort 2 comprises 45 children 2-8 years old. Thirty of them will receive one dose of the vaccine at a dose of 10^8 $TCID_{50}$ and 15 will receive one dose of placebo. Sites will enroll participants into Cohorts 1 and 2 simultaneously. Once 25 or more participants in each of the first 2 cohorts (Cohorts 1 and 2) have completed Day 8, the SRC will evaluate if any halting rules are met and if it is deemed safe, enrollment in Cohort 3 may open. Enrollment within each cohort will not halt during the SRC review. Cohort 2 must fully enroll before enrollment in Cohort 3 may begin. If any halting rules are met or if the SRC raises any safety concerns other than halting rules that should be independently reviewed, the external Safety Monitoring Committee (SMC) will meet to discuss the data prior to progression to the next cohort.

Cohort 3 consists of 25 children 2-8 years old. Fifteen of them will receive one dose of vaccine at 10^9 $TCID_{50}$ and 10 will receive one dose of placebo. Once all 25 participants in Cohort 3 have completed Day 8 of follow-up, similar to Cohorts 1 and 2, the SRC will review to ensure no

halting rules are met and if no rules are met and the SRC determines it is safe to proceed, simultaneous enrollment into Cohorts 4 and 5 can begin. If any halting rules are met or any concerns are raised by the SRC, an external SMC may meet to discuss the data for recommendations on either progression or clinical trial modification before progression to the next cohort.

Cohort 4 consists of 25 children 2-8 years old; 15 of them will receive two doses of vaccine at 10^9 $TCID_{50}$ and 10 will receive two doses of placebo, with a 28-day interval between the first and second doses. Cohort 5 will enroll 8 influenza naïve children (defined as children without receipt of influenza vaccine and without previous documented influenza infection) who are 6-23 months old who will be randomly assigned to receive two doses of 10^7 $TCID_{50}$ Sing2016 M2SR (n=6) or two doses of placebo (n=2) with a 28-day interval between the first and second dose. Once all 8 participants in Cohort 5 have completed Day 8 of follow-up after the first dose, the SRC will conduct a safety assessment before beginning enrollment in Cohort 6. Once 6 of the 8 participants in Cohort 5 have completed Day 4 after the second dose (e.g., Study Day 32), the following process will be implemented prior to receipt of the second dose for any participant in Cohort 6:

1. An email will be sent from the site to the SDMCC attesting that all data entry through Day 4 after the second dose (e.g., Study Day 32) for at least 6 participants in Cohort 5 has been completed.
2. The SDMCC will perform data cleaning as it pertains to assessment of the halting rules.
3. The SDMCC will assess if there were any halting rules met based on data entered in the data system and email DMID indicating what halting rules, if any, were met based on data entered in the data system.
    a. If any of the halting rules were met, the study will halt and a review by the SMC, sponsor, and investigators will convene as specified in Section 7.1.
    b. If none of the halting rules were met, DMID will confirm that the study may proceed as per-protocol.

Cohorts 6 and 7 will also enroll 6 to 23-month-olds who are influenza-naïve. Cohort 6 will consist of 26 children. A lead-in group of 8 children will be randomly assigned to receive either two doses of 10^8 $TCID_{50}$ of the Sing2016 M2SR (n=6) or two doses of placebo (n=2). Once the first 8 children have completed Day 8 of follow up after the first dose, the SRC will review the safety and determine if Cohort 7 may open to enrollment. Cohort 7 enrollment will not begin until Cohort 6 is fully enrolled. Similar to other cohorts, the additional 18 children in Cohort 6 may continue to enroll during the SRC review.

Cohort 7 will be the final cohort. 26 children will be randomly allocated to receive two doses of either 10^9 $TCID_{50}$ Sing2016 M2SR (n=18) or placebo (n=8). This cohort does not have a "lead-in" group since data from such a group are not needed to allow the enrollment in a subsequent cohort. However, the study-wide and individual halting rules still apply.

For all cohorts, a minimally acceptable pre-vaccination blood volume is set at one 5-mL tube of blood (SST for serum), regardless of age (Appendix A – Blood and Fluid Collection Volumes by

age group). Should the site not be able to collect that blood before the first vaccination, the child will not be permitted to continue.

After the administration of the above noted vaccine doses, safety will be assessed by the evaluation of reactogenicity from Days 1-8 for participants in Cohorts 1-3, who are receiving a single dose of vaccine or placebo. For Cohort 4 – 7, who receive two doses of vaccine or placebo, safety assessment will be from Day 1-8 and approximately Days 29-36. All participants will be followed for AEs, AESIs, NOCMCs, and SAEs in the time periods described in the protocol. Safety will be reviewed by the internal SRC and an external independent SMC, as applicable. The responsibility of the SRC will be to review if any halting rules are met and to determine if safety data available permit progression from cohort to cohort. The responsibility of the SMC will be to review safety outcomes if criteria for progression to a subsequent cohort are not met, a halting rule occurs, or as otherwise determined in their charter. The SMC may propose to the sponsor and investigators to make study product dose adjustments or other modifications. The roles of the SRC, and SMC are described in detail below. All participants will be evaluated for immune responses as described below.

All participants will be vaccinated with the first dose of investigational product or placebo in the influenza off-season for the Northern Hemisphere. In the past, we could reliably predict that the influenza off-season would begin by May in the regions where this study will be conducted. Since the start of the COVID-19 pandemic, the seasonality of influenza in the US has become less predictable, and the influenza off-season may begin as early as March 1. The decision for each site's start either on or after March 1 will be determined by the protocol team and sponsor, using data from local (e.g., site-affiliated hospital/healthcare), state, or regional microbiological surveillance systems representative of the recruitment catchment area of each site. The decision(s) for determination of influenza off-season also may consider data from larger surveillance programs, such as CDC [7]. Each site may begin enrolling as early as March 1, if influenza virus is reported in less than 5% of respiratory specimens, and absolute numbers of influenza cases are consistent with off-season status. Study activities may be halted if the proportion of local or regional respiratory specimens positive for influenza virus exceeds 10% and the local or regional ILI activity exceeds low. Sites may re-initiate enrollment activities if the enrollment criteria are met once again for two consecutive weeks.

During the Fall (September-November), participants will receive CDC recommended seasonal influenza vaccine (IIV4) administered at least 28 days after receipt of the final investigational vaccine administration. The final study visit for each participant will occur in April of the following calendar year.

Due to the limited availability of product, and funding to support additional years of enrollment into Cohorts 5, 6, and 7, the decision was made to stop enrollment after the final participant was enrolled into Cohort 4.

Study Objectives:

Primary

1. To assess the safety and tolerability of one and two administrations of the Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

Secondary

2. To assess the humoral immunogenicity (serum antibody and mucosal antibody responses) directed against homologous viral strains after one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

Exploratory

3. To assess the cellular immunogenicity (T-cell immune responses) against Sing2016 M2SR H3N2 influenza vaccine following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
4. To assess participant immunological response to neuraminidase (NA) following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
5. To assess the humoral immunogenicity (serum and/or plasma antibody) and mucosal antibody responses and cellular immunogenicity (T-cell responses) after seasonal influenza vaccine (IIV4) following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
6. To assess mucosal antibody responses directed against homologous viral strains after one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
7. To assess humoral immunogenicity (serum and/or plasma antibody), and mucosal antibody responses directed against heterologous viral strains of one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
8. To conduct additional characterization of humoral immunity against homologous and/or heterologous viral strains (e.g., extra-neutralizing antibody function) following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
9. To assess the effects of age, dose level, sex, prior receipt of seasonal influenza vaccine(s), and other variables on humoral immunogenicity (serum antibody and mucosal antibody responses) directed against homologous viral strains of one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

Study Endpoints:

Primary

1. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience solicited local reactogenicity events, of all severity grades and by grade, in the 7 days following administration of each dose of vaccine

2. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience solicited systemic reactogenicity events, of all severity grades and by grade, in the 7 days following administration of each dose of vaccine
3. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience unsolicited non-serious adverse events, of all severity grades and by grade, in the 28 days following administration of each dose of vaccine
4. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience adverse events of special interest (AESIs) the time of first vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)
5. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience Serious Adverse Events (SAEs) from the time of first vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)
6. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience new-onset chronic medical conditions (NOCMCs) from the time of the first study vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)

Secondary

7. The number and percentage of participants in Cohorts 1, 2, and 3 with putative seroprotection at baseline (Day 1) and on approximately Day 29, defined as an HAI titer ≥1:40 in serum against an H3N2 M2SR-like virus
8. The number and percentage of participants in Cohort 4 with putative seroprotection at baseline (Day 1) and on approximately Day 57, defined as an HAI titer ≥1:40 in serum against an H3N2 M2SR-like virus
9. The number and percentage of participants in Cohorts 1, 2, 3 with neutralization titer ≥1:40 in serium against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29
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19. Mean secretory IgA (sIgA) response, as measured by binding antibody multiplex (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 before vaccination and on approximately Day 29
20. Mean secretory IgA (sIgA) responses, as measured by binding antibody multiplex (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 4 before vaccination and on approximately Day 57
21. Mean change (difference) from baseline in secretory IgA (sIgA) response, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
22. Mean change (difference) from baseline in secretory IgA (sIgA) response, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 4 on approximately Day 57

Exploratory

23. Antigen-specific T-cell responses to vaccine immunogens, at baseline (Day 1) and following one or two doses of vaccine
24. Humoral antibody, as detected by one or more assays that measure responses to NA, at baseline (Day 1) and following one or two doses of vaccine or IIV4
25. Serum antibody, as detected by HAI and neutralization, directed against homologous H3N2 strains measured at baseline (Day 1) and after IIV4 administration
26. Antigen-specific T-cell responses to vaccine immunogens, at baseline (Day 1) and measured after IIV4 administration
27. Serum antibody to additional influenza antigens, as detected by multiplex ELISA assays and/or protein microarrays with subsequent evaluation of HAI and neutralization when multiplex ELISA screen is positive, directed against heterologous strains at baseline (Day 1) and following one or two doses of Sing2016 M2SR H3N2 vaccine
28. Humoral and/or mucosal antibody neutralizing and extra-neutralizing function against homologous and heterologous strains based on positive responses detected by methods described above.
29. Associations between serum antibody responses and age, dose level, sex, prior receipt of seasonal influenza vaccine(s) and other variables over time.
30. Associations between mucosal antibody responses and age, dose level, sex, prior receipt of seasonal influenza vaccine(s) and other variables over time.
31. Geometric mean titer (GMTs) of plasma HAI against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29 for participants in Cohorts 1, 2 and 3
32. GMTs of plasma HAI against an H3N2 M2SR-like at baseline (Day 1) and on approximately D57 for participants in all Cohorts 4
33. Geometric Mean Titers (GMTs) of plasma neutralizing antibodies against an H3N2

M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1) and on approximately Day 29

34. Geometric Mean Titers (GMTs) of plasma neutralizing antibodies against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1) and on approximately Day 57
35. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma HAI titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
36. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma HAI titers against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
37. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma neutralization titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
38. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma neutralization titers against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
39. The number and percentage of participants in Cohorts 1, 2, and 3 with putative seroprotection at baseline (Day 1) and on approximately Day 29, defined as a plasma HAI titer ≥1:40 against an H3N2 M2SR-like virus
40. The number and percentage of participants in Cohort 4 with putative seroprotection at baseline (Day 1) and on approximately Day 57, defined as a plasma HAI titer ≥1:40 against an H3N2 M2SR-like virus
41. The number and percentage of participants in Cohorts 1, 2, 3 with plasma neutralization titer ≥1:40 against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29
42. The number and percentage of participants in Cohort 4 with plasma neutralization titer ≥1:40 against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 57
43. Geometric mean titers (GMTs) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29
44. Geometric mean titers (GMTs) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
45. Geometric mean fold rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
46. Geometric mean fold rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57

47. Mean total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29
48. Mean total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
49. Mean change (difference) from baseline in total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
50. Mean change (difference) from baseline in total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
51. Mean normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29
52. Mean normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
53. Mean change (difference) from baseline in normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
54. Mean change (difference) from baseline in normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
55. Geometric Mean Titers (GMTs) of plasma or serum NA antibodies against an H3N2 NA for participants in Cohorts 1, 2, and 3 at baseline (Day 1) and on approximately Day 29
56. Geometric Mean Titers (GMTs) of plasma or serum NA antibodies against an H3N2 NA for participants in Cohort 4 at baseline (Day 1) and on approximately Day 57
57. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma or serum NA antibodies against an H3N2 NA for participants in Cohorts 1, 2, and 3 on approximately Day 29
58. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma or serum NA antibodies against an H3N2 NA for participants in Cohort 4 on approximately Day 57
59. The number and percentage of participants in Cohorts 1, 2, 3 with NA antibody titer ≥1:40 against an H3N2 NA at baseline (Day 1) and on approximately Day 29
60. The number and percentage of participants in Cohort 4 with NA antibody titer ≥1:40 against an H3N2 NA-like virus at baseline (Day 1) and on approximately Day 57

Eligibility:

A potential participant is eligible to join this trial if he/she meets all the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria:

1. Participant is a male or female child aged 6 months to 17 years inclusive at time of enrollment (each cohort has its own age upper and lower limits[1])
   [1]Cohort 1: 9-17 years (on or after the 9th birthday and before the 18th birthday at the time of the first dose); Cohorts 2, 3, and 4: 2-8 years (on or after the 2nd birthday and before the 9th birthday at the time of the first dose); Cohorts 5, 6, and 7: 6 months to 23 months (on or after the 6th month of life based on calendar day and before the second birthday at the time of the first dose)
2. For Cohorts 1 to 4, receipt of at least 2 doses of seasonal influenza vaccine in the past.
3. For Cohorts 5 to 7, receipt of no seasonal influenza vaccines in the past and no documented history of laboratory-confirmed influenza illness
4. Parent/guardian of the participating child provides written informed permission and participating child provides assent[2] prior to initiation of any study procedures
   [2]as appropriate by age or development and approved by the IRB
5. Parent/guardian and participant, as appropriate, are able to understand and comply with planned study procedures and are available for all study visits
6. Participant is in good health as assessed by the principal investigator or other designated study investigator[3]
   [3]based on medical history and physical examination (physical examination may be done as part of routine medical care or specifically for eligibility screening)
7. Parent/guardian of the participating child agrees not to allow the participant to join another clinical trial that includes an investigational agent or device during the study period
8. A female participant of child-bearing potential[4] agrees to abstain from sexual intercourse or to correctly use an acceptable method of contraception[5]
   [4]A female of child-bearing potential is defined as a female who is post-menarchal and not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure. This applies only to participants in Cohort 1.
   [5]Acceptable methods of contraception must be used from 30 days prior to vaccination until 60 days after the last study vaccination (not IIV4) and include full abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more or shown to be azoospermic prior to the participant receiving the study vaccination, barrier methods such as condoms or diaphragms/cervical cap, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives ("the pill").
9. A female participant of child-bearing potential[4] must have a negative urine pregnancy test within 24 hours prior to each study product
10. A male who is sexually active with a female of childbearing potential[4] must agree to use an acceptable method of contraception[6]
    [6]From the time of the first dose of study vaccine until 60 days after receipt of the last dose study vaccine, only in Cohort 1. The only acceptable method of contraception for males who are sexually active with females of childbearing potential is condoms.

Exclusion Criteria:

1. Has a body temperature of 38.0°C/100.4°F (oral or axillary) or greater or another acute illness[7] within the 72 hours prior to study vaccination
   [7]Potential participants who are recovering from an acute illness and have residual minimal symptoms, which, in the opinion of the site principal investigator or appropriate sub-investigator, will not likely affect the evaluation of outcome measures are not ineligible. Temperature evaluation will not be performed as a study procedure on participants prior to administration of seasonal influenza vaccine
2. Has any medical or mental health disease or condition[8] that would render study participation unsafe, or would interfere with the evaluation of the responses
   [8]in the opinion of the site principal investigator or appropriate sub-investigator
3. Has a history of provider-diagnosed asthma requiring the use of medications at any age, or has had a wheezing episode or use of medications to treat asthma in the 12 months prior to screening.
4. Has immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy
5. Has a diagnosis of or history of malignant neoplastic disease
6. Has taken oral, parenteral (intramuscular or intravenous), inhaled, or nasal corticosteroids of any dose within 30 days prior to study vaccination
7. Has known HIV, hepatitis B, or hepatitis C infection
8. Has known hypersensitivity or allergy to any components of the study vaccine or material in the nasal delivery device[9]
   [9]vaccine components: sucrose, sodium chloride, phosphate, glutamate; delivery device material: polycarbonate, polypropylene, synthetic rubber
9. Has a history of severe reactions following previous immunization with licensed or unlicensed influenza vaccines
10. Has a history of an anatomic disorder of the nares or nasopharynx
11. Has a history of chronic sinus infections
12. Has a history of or currently smokes or vapes
13. Has a history of Guillain-Barré syndrome
14. Use of aspirin- or salicylate-containing products in the 30 days prior to or intends to use these products in the 30 days following administration of the investigational vaccine
15. Has a history of documented influenza or receipt of influenza antiviral treatment in the 4 months prior to the first vaccination
16. Receipt of any antiviral drug within the week prior to or following the investigational vaccine
17. Receipt of a licensed live vaccine within 30 days prior to the first study vaccination, or plans to receive a licensed live vaccine within the 30 days after the last study vaccination
18. Receipt of licensed inactivated non-influenza vaccine[10] within 14 days prior to the 1st study vaccination, or plans to receive licensed, inactivated vaccine within the 30 days after the last study vaccination
    [10] Participants will be asked to avoid receipt of any routine licensed vaccines or vaccines under emergency use authorization during the periods described.
19. Receipt of an influenza vaccine within the 4 months prior to the first study vaccination or plans to receive an influenza vaccine following the last study vaccination. Seasonal IIV4 will be received by participants as part of this trial.
20. Receipt of immunoglobulin or other blood products within the 6 months prior to the first study vaccination or plans to receive during the period of study participation.
21. Receipt of an experimental[11] agent or device within the 6 months prior to the first study vaccination or expects to receive an experimental agent or device during the study period

[11]Products for treatment or prevention of COVID-19, when received under Emergency Use Authorization [EUA] or full FDA approval and not as part of a clinical trial, will not be deemed "experimental" for the purposes of this criterion and will not make an otherwise eligible prospective participant ineligible.

22. Is a family member of study personnel or personnel directly involved in the conduct or monitoring of the study
23. Receipt of an approved or experimental product for treatment or prevention of COVID-19 within the 10 days[12,13] prior to study enrollment

    [12]Participants may enroll if greater than 10 days after receipt of the COVID-19 treatment or prevention.
    [13]Participants who are receiving COVID-19 vaccines around the time of dosing of the investigational product will be asked to avoid COVID-19 vaccination within the 10 days before any vaccination in the study and within any reactogenicity period (the day of and 7 days following each intranasal vaccination).
24. Inability of the study team to collect 5 mL of blood from the participant before the first vaccination (pre-vaccination blood).

Study Phase: Phase 1b

Study Population: Healthy 6 month to 17-year-old children, inclusive

Sites: 4 US sites

Study Intervention:
The study intervention is intranasal vaccination with Sing2016 M2SR H3N2 influenza vaccine supplied by FluGen, Inc. Commercially available saline will be used as the corresponding placebo. The Sing2016 M2SR H3N2 influenza vaccine made by FluGen is a live vaccine product with a defective M2 gene which renders the virus unable to replicate and generate progeny in animals and in humans, making its infectivity self-limiting. This attenuation is expected to improve safety while maintaining the ability to elicit an immune response. Furthermore, like FluMist®, the Sing2016 M2SR H3N2 vaccine induces both a mucosal and cell-mediated immune response and is administered intranasally. However, unlike FluMist®, the Sing2016 M2SR H3N2 vaccine has been demonstrated to induce Immune responses in animal models even in the presence of pre-existing antibodies. Early studies of this study intervention have reported no serious adverse events (SAEs), adverse events (AEs) of significance or any halting rules. Further, Sing2016 M2SR H3N2 induces antibodies in animal models against the highly conserved stem region of hemagglutinin (HA) as well as against NA glycoproteins on the surface of the influenza virus. In this study we evaluate the safety and reactogenicity of the Sing2016 M2SR H3N2 influenza vaccine in a dose-escalating and age de-escalating double-blinded randomized manner.

All enrolled children will also receive seasonal influenza vaccine with licensed IIV4 at the dose and schedule recommended for the individual participant with first dose administered once seasonal influenza vaccine is available usually between September through November but no later than end of November of the same calendar year.

The study is designed as a Phase 1b, randomized, double-blind, dose-escalating, age de-escalating, placebo-controlled study of approximately 200 children ages 6 months to 17 years. Cohort 1 to Cohort 4 will enroll non-naïve children. Cohorts 5-7 will enroll 6- to 23-month-olds who are influenza-naïve (no previous influenza vaccine and no previous documented influenza

infection). Safety reviews may be performed by the SRC, and the SMC. Details on those reviews are found the protocol.

Total Study Duration: Up to 4 years

Participant Duration: Up to 14 months

Safety: The study has three sets of halting criteria.

Halting Criteria Study-wide:
The study-wide halting criteria apply to all enrolled participants study-wide and serve to pause enrollment in the study until the data are reviewed by the SMC, sponsor, and investigators. The criteria are defined in the protocol.

Halting Criteria for "Lead-In" Participants:
The 8 participants in Cohort 5 and the first 8 participants in Cohort 6 are termed "lead-ins" because data on safety and tolerability from Days 1 to 8 from these participants will inform enrollment into subsequent cohorts. For progression to Cohort 6, safety data must be reviewed for Cohort 5 participants through Day 8 of follow-up after the first dose. Given that the lead-ins are young (6-23 months), influenza-naïve, and only 8 participants in each of those cohorts, the halting criteria are slightly more stringent with lower thresholds for tolerability of AEs and SAEs. The criteria are defined in the protocol.

Halting Criteria for Individual Participants:
These halting criteria will apply to any individual participant within the study. If any of these halting criteria are met, the individual will not receive any additional doses of investigational product.

Safety Committee Reviews:
The study has three safety review persons or groups, as defined below.

SRC:
The Safety Review Committee (SRC) will review the blinded data required to allow progression from cohort to cohort. The review and the composition of this committee are detailed in the protocol. Ad hoc reviews may also be called for by the investigators or sponsor. Decisions are dependent on the failure to meet halting criteria when the required follow-up data are available.

SMC:
The Safety Monitoring Committee (SMC) is an independent group that will provide safety oversight for the study. They will meet to review safety data as delineated in their charter. They will meet and make recommendations if any halting/pausing rules, as listed above, occur. They will also meet if the SRC decides not to permit progression to a subsequent cohort or requests the SMC meet to make that determination. The SMC may also call ad hoc meetings. The SMC deliberations may occur by teleconference, videoconference, or email.

Detailed description of the role of each of the levels of safety review can be found in Section 10.1.6.

1.2 Schedule of Activities (SoA)

Table 1: Schedule of Activities:

Visit number	*0*[1]	*1*[1]	*2*	*3*	*4*	*4A*[2]	*4B*[2]	*4C*[2]	*5*	*6*	*7*
Target day	*-*	*1*	*3*	*8*	*29*	*V4+3d*	*V4+7d*	*V4+28d*	*-*	*V5+28d*	*April*
First day of window	*-28*	*1*	*3*	*8*	*26*	*V4+3d*	*V4+7d*	*V4+25d*	*09.01*	*V5+25d*	*04.01*
Final day of window	*-1*	*1*	*4*	*11*	*32*	*V4+4d*	*V4+10d*	*V4+31d*	*11.30*	*V5+31d*	*04.30*
Visit Type	*Screen*	*Vaccine*	*Follow*	*Follow*	*Vaccine/ Follow*	*Follow*	*Follow*	*Follow*	*IIV4*	*Follow*	*Follow*
Visit Venue	*Clinic*	*Clinic*	*Phone*	*Phone*	*Clinic*	*Phone*	*Phone*	*Clinic*	*Clinic*	*Clinic*	*Phone*
Procedures											
Informed consent[3]											
Collect demographics[4]											
Medical history, eligibility[5]											
Concomitant med review[6]											
Physical exam[7]											
Pregnancy test[8]											
Randomization[9]											
Administer study product[10]											
Administer IIV4[11]											
Solicited AE[12]											
Non-serious unsolicited AEs[13]											
SAEs/AESIs/NOCMCs[14]											
Blood for immune response[15]											
Nasal wash immune response[16]											

Color legend: Gray= all cohorts, Orange = only Cohorts 1, 2, and 3 (cohorts receiving single dose of IP); Blue= only Cohorts 4, 5, 6, 7 (cohorts receiving 2 doses of IP); Green = only Cohort 1

[1]Screening and enrollment may occur on the same day (Day 1) or up to 28 days apart. Consent is obtained either on the screening day (when there is a separate screening day), or on the enrollment day (if there is no separate screening day). If there is a separate screening day, then all the procedures noted for that day will be performed on the screening day and the following will be done on the enrollment day (Day 1): update medical history and update concomitant medications. A physical will be performed either on the screening day or the enrollment day but is not required on both days. If performed on the screening day, a targeted physical exam may occur on the enrollment day, if needed, but is not required. Pregnancy test, final eligibility determination, randomization, and administration of study product occur on Day 1.

[2]Visits 4A, 4B, and 4C will not be done for Cohorts 1, 2, and 3 because participants in these cohorts receive a single dose of vaccine or placebo. Participants in Cohorts 4, 5, 6, and 7 are scheduled to receive 2 doses, but if they discontinue participation prior to the second dose, these participants will not complete Visits 4A, 4B, and 4C rather will follow the visit schedule of Cohort 1,2 and 3.

[3]Prior to any study related procedures, families will be provided information about the study and informed consent will be obtained from the parent/guardian and assent obtained from the participant, when required by the IRB. Note that the screening and enrollment may occur on the same day (Day 1) or up to 28 days apart. Consent is obtained either on the screening day (when there is a separate screening day), or on the enrollment day (if there is no separate screening day).

[4]The investigator or appropriately delegated study staff will obtain demographic data.

[5]The investigator or appropriately delegated study staff will obtain medical history and information necessary to determine eligibility. Since screening may occur up to 28 days before enrollment, if eligibility and medical history are taken at a screening visit, they will be updated on the day of enrollment, before randomization. Demography will not be updated. Review of eligibility criteria will occur before second dose of the study product.
[6]Medications taken by the participant in the 30 days before enrollment will be recorded (while all medications taken from 30 days prior to screening, whether on the same day as enrollment or not, will be solicited). At subsequent visits, until Visit 4 (Day 29) for Cohorts 1, 2, and 3 and until Visit 4C (target Day 57, or 28 days after second dose), for Cohorts 4, 5, 6, and 7, updates to previous concomitant medications and additional medications taken in the interim will be recorded. No concomitant medications will be recorded after Day 57.
[7] Physical examination will occur for all participants at either Visit 0 or Visit 1. If they receive it at Visit 0, they may get a targeted physical exam on Visit 1 if necessary, to determine eligibility. Participants may receive an optional targeted physical exam at any other clinic visits at investigator discretion.
[8]Urine pregnancy test will be performed for females of childbearing potential and recorded negative within the 24 hours prior to each study product administration. No pregnancy test will be done before the seasonal influenza vaccine administration.
[9]Participants will be randomly allocated to receive either vaccine or placebo. For those receiving 2 doses (Cohorts 4, 5, 6, and 7), they will receive the same product (vaccine) at the specified dose or placebo, at both time points.
[10]Participants in Cohorts 1, 2, and 3 will be randomly allocated to receive on Day 1 a single dose of intranasal 2016 SingM2SR H3N2 vaccine or placebo. Participants in Cohorts 4, 5, 6, and 7 will be randomly allocated to receive 2 doses of intranasal 2016 SingM2SR H3N2 vaccine or placebo. The first dose will be given on Day 1 and the second, when applicable, on approximately Day 29. In order to receive the second dose, participants in Cohorts 4, 5, 6, and 7 must meet the eligibility requirements for subsequent dosing, as described in the protocol.
[11]All participants in Cohorts 1, 2, 3, and 4 (non-naives) will be provided a single injection of licensed, seasonal influenza vaccine (IIV4) in the period from September to November in the same year but at least 28 days after they receive the investigational vaccine or placebo. If vaccine is available earlier than September 1, and the participant is at least 28 days since the last intranasal vaccine, he/she may receive it earlier than September 1. Participants in Cohorts 5, 6, and 7 (naives) will receive 2 doses of IIV4, as currently recommended per standard of care. (Should the standard recommendations change, participants will receive the updated standard of care.) This vaccine will be given no earlier than 28 days following the final administration of the experimental vaccine or placebo. No safety or reactogenicity data related to IIV4 will be collected. Participants in Cohorts 1-3 (who receive a single dose of intranasal 2016 SingM2SR H3N2 vaccine or placebo) may receive their IIV4 at Visit 4 and participants in Cohorts 4-7 (who receive 2 doses of intranasal 2016 SingM2SR H3N2 vaccine or placebo) may receive their IIV4 at Visit 4C. IIV4 will not be given until after the completion of other study procedures. Participants may also receive their COVID-19 study product on the same day as their IIV4.
[12]Solicited AEs will first be collected 20 minutes after administration of vaccine or placebo (immediate reactogenicity). Additional solicited AEs will be collected by memory aid (eDiary) on Days 1-8 for participants in all cohorts and additionally on the 7 days following the second vaccination (e.g., Days 29-36 for those receiving the second vaccination on the target Day 29). Solicited AEs consist of immediate reactogenicity, local reactogenicity (upper respiratory

symptoms as defined in the protocol) and systemic reactogenicity (constitutional signs and symptoms as defined in the protocol). Reactogenicity assessments are based on age at enrollment.

[13]Non-serious unsolicited AEs will be collected and recorded from Day 1 after administration of vaccine or placebo for all participants until Day 29 and for an additional 28 days after the second vaccination (Day 57 for those who receive the second vaccination on Day 29) for participants in Cohorts 4, 5, 6, and 7.

[14]SAEs, AESIs, and NOCMCs, as defined in the protocol, will be collected from Day 1, following vaccination, until the end of study follow-up, the date in April of the calendar year following enrollment at which Visit 7 occurs. The only AESI is wheezing. Participants will be evaluated for wheezing episodes in 2 phases. For the day of each vaccination and the next 28 days, active surveillance will occur, and each episode will require a standardized clinical evaluation as defined in Appendix B – Evaluation Criteria for Acute Wheezing. After 28 days from the last receipt of investigational product, parents will be instructed to notify the research team and recording of the relevant information may be completed by medical record review or parental reported outcomes.

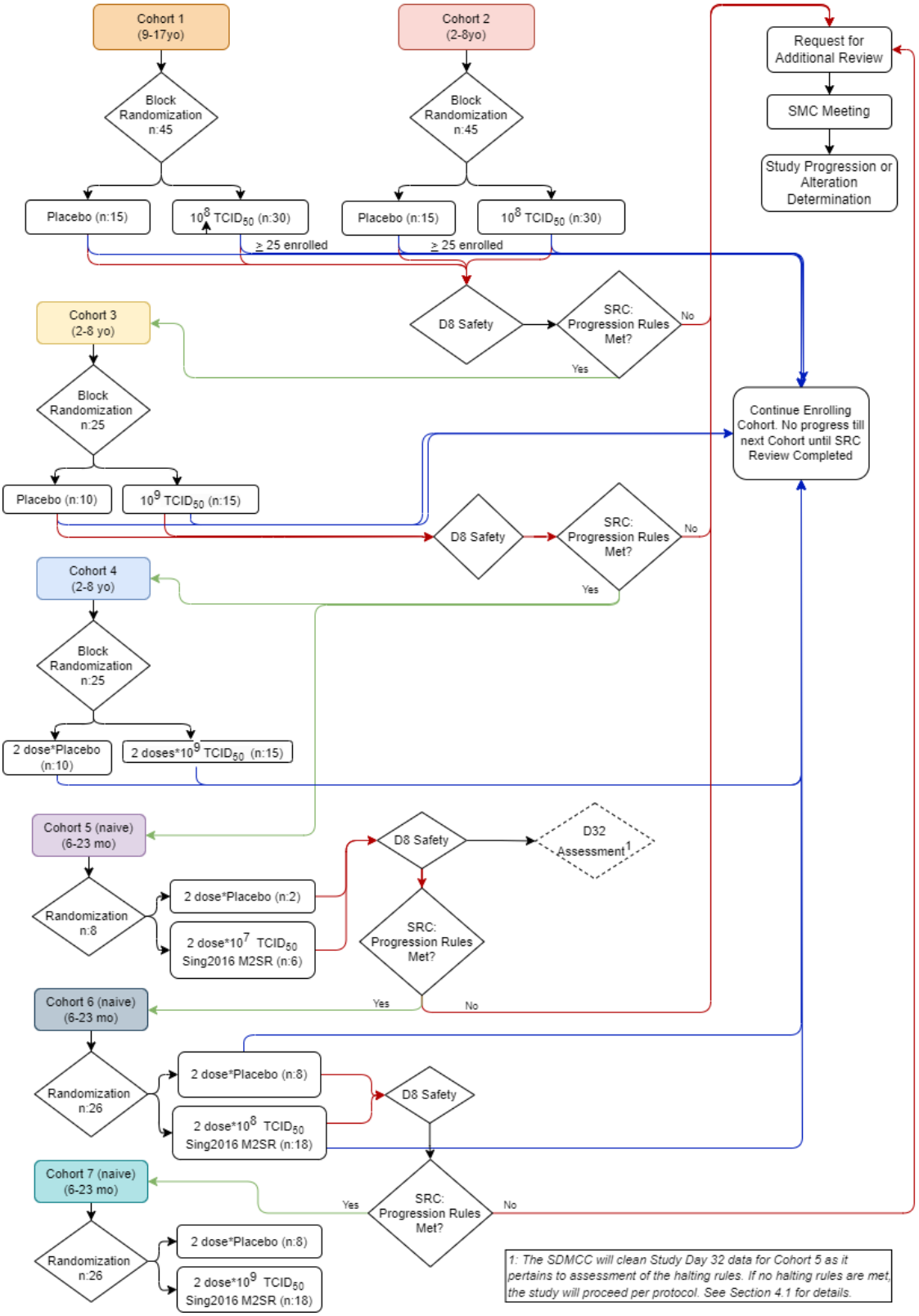
[15]All participants will have blood drawn for immune responses at 3 times: on Day 1, before vaccination, for all participants; on Day 29, for participants in Cohorts 1, 2, and 3; 28 days after second vaccination (Day 57 for participants who receive their second vaccination on Day 29), for participants in Cohorts 4, 5, 6, and 7; and approximately 28 days after receipt of the licensed, seasonal influenza vaccine (IIV4), for all participants. The assays performed and blood volumes and tubes required are found in the protocol and MOP. The volumes and number of tubes collected are noted in Appendix A – Blood and Fluid Collection Volumes by age group.

[16]All participants will have nasal washes collected for mucosal immune responses at 3 times: on Day 1, before vaccination, for all participants; on Day 29, for participants in Cohorts 1, 2, and 3; approximately 28 days after second vaccination (Day 57 for participants who receive their second vaccination on Day 29), for participants in Cohorts 4, 5, 6, and 7; and approximately 28 days after receipt of the licensed, seasonal influenza vaccine (IIV4), for all participants. The assays performed and samples required are found in the protocol and MOP. The volumes and number of tubes collected are noted in Appendix A – Blood and Fluid Collection Volumes by age group.

1.3 Study Schema

Figure 1– The overall study design is captured in the schema below:

Note: Due to the limited availability of product, and funding to support additional years of enrollment into Cohorts 5, 6, and 7, the decision was made to stop enrollment after the final participant was enrolled into Cohort 4.



2. INTRODUCTION

2.1 Study Rationale

The CDC estimates 7,000 to 26,000 children are hospitalized each influenza season in the US due to illness caused by influenza viruses. Deaths associated with influenza in US persons from birth to 17 years of age were first reportable in 2004. In the 17 years since then, the frequency of reported influenza-associated deaths in US children has ranged from 37, in the 2011-2012 season, to 196 deaths, in the 2019-2020 season (The final reports for the 2020-2021 season are not available at the time the protocol was written, but as of March 18, 2021, there was only 1 reported influenza-related pediatric death in the US). Because not all influenza-associated deaths in children are reported, CDC also uses models to estimate the actual numbers of influenza-related deaths. They estimate as high as 1200 pediatric deaths per year, using these models [8, 9].

Vaccination remains the best strategy for preventing influenza and its associated morbidity and mortality in young children[10]. Currently available inactivated and recombinant influenza vaccines primarily aim to induce neutralizing antibodies directed towards the hemagglutinin (HA) region of the influenza virus. No cellular immune response or broad cross-reactive (heterosubtypic) immunity against divergent strains is induced by current vaccines. Therefore, current influenza vaccine seasonal efficacy is strongly dependent on a close match between circulating viruses and neutralizing antibodies. This phenomenon was demonstrated by an efficacy of just 13% in the 2014-2015 influenza season, a year when the frequency of pediatric mortality was estimated to be 600 in the US[11].

FluGen, Inc. has developed a novel M2SR (M2-deficient Single Replication) intranasal live attenuated influenza vaccine platform. Unlike the currently licensed live attenuated influenza vaccine platform, the M2SR vaccine platform does not express one of the essential viral matrix proteins (influenza M2), thus restricting it to a single replication cycle in the host. Pre-clinical testing of the M2SR platform-based vaccines has demonstrated broader reactivity against multiple influenza subtypes and heterosubtypes. Furthermore, even in the presence of pre-existing anti-influenza immunity, a systemic, cellular, and mucosal immune response has been noted following a single intranasal dose of an M2SR-platfomr vaccine [5, 12]. In a pre-clinical proof-of-concept study, a FluGen vaccine, Bris10 M2SR H3N2 vaccine (clade 1), which uses the same platform but different source for HA and NA than found in Sing2016 M2SR H3N2 vaccine, provided protection to ferrets against a drifted H3N2 challenge virus, A/Alaska/140/2015, a virus belonging to clade 3c.2a1[5].

Further, some studies indicate that horizontal transmission of vaccine viruses may, very infrequently, occur with the live, attenuated influenza vaccine (LAIV) currently licensed in the US (FluMist®). This property has led to a preference for IIV in persons who care for or are close contacts with severely immunosuppressed persons who require a protective environment and that those who receive LAIV and have such contacts should avoid contact with such persons for 7 days after receipt of LAIV. Given the M2 protein defect in the Sing2016 M2SR H3N2 vaccine, viral progeny are not generated, lending an additional benefit to this approach [4]. This has been confirmed in early *in vitro* and *in vivo* studies in ferrets and subsequently in healthy adults (total of ~280 participants in three studies).[13] Consequently, no special precautions will need to be taken in any subsequent trial of an M2SR-based vaccine to exclude participants who have household or other close contacts with immunosuppressive conditions.

Lastly, an additional potential benefit of vaccines in the M2SR-platform is that they may, due to their inability to replicate in humans, lead to a reduced risk of post-vaccination wheezing when compared to live attenuated influenza vaccines. If true, the indications for vaccines using this platform may potentially allow for children with asthma.

The above noted early successes of the M2SR vaccine platform and potential improvements over current influenza vaccines warrant further investigation of Sing2016 M2SR H3N2 vaccine in participants 6 months to 17 years of age.

2.2 Background

2.2.1 Purpose of Study

The purpose of this study is to assess the safety, tolerability/reactogenicity, and immunogenicity of the FluGen Sing2016 M2SR H3N2 vaccine in children aged 6 months to 17 years. This will be done in a Phase 1b, double blinded, age de-escalating, dose-escalating fashion where in each cohort, participants will be randomly assigned to receive either vaccine or placebo, as described in detail under study design. All participants will be followed for tolerability or reactogenicity, safety, and immune responses.

2.3 Risk/Benefit Assessment

2.3.1 Known and Unknown Potential Risks

The potential risks of participation in this trial are those associated with administration of Sing2016 M2SR H3N2 vaccine, placebo, and the licensed inactivated influenza vaccine (IIV4), having blood drawn, receiving an intramuscular injection or intranasal spray, having a nasal wash or swab procedure performed, the potential risks to a fetus of a participant who inadvertently becomes pregnant, breach of confidentiality, and unknown risks.

2.3.1.1 Potential Risks Associated with Receipt of the Vaccine or Placebo

Sing2016 M2SR H3N2 Vaccine:

The risks of receiving the intranasal live attenuated influenza vaccines include risks at the site of administration (local, in and around the nose) and systemically (other places in the body).

Based on available safety data, the safety profile of Sing2016 M2SR is similar to other LAIV vaccines, such as FluMist®. Possible local side effects of the Sing2016 M2SR vaccine include edema, dryness, pain/irritation, congestion, rhinorrhea, sneezing, and nasal bleeding. In other local tissues, they may be associated with sore throat (pharyngitis), difficulty swallowing, ear infections, conjunctivitis, itchy eyes, and sinusitis. Similar to other LAIVs, these effects may be more pronounced in seronegative individuals. Systemic side effects may include fatigue and headaches and seronegative individuals may also present with low-grade fever. No SAEs have been reported previously from administration of the Sing2016 M2SR vaccine. Following administration of other LAIVs, pulmonary adverse effects, including wheezing, have been reported. Given that Sing2016 M2SR H3N2 influenza vaccine cannot replicate to spread cell to cell, adverse events are not expected to be more severe or frequent than replicating LAIV vaccine.

Horizontal transmission of the live, attenuated replicating influenza vaccine virus found in FluMist® may infrequently occur. However, given that M2SR is replication deficient, horizontal transmission is not expected and has not occurred to date. *In vitro* and *in vivo* studies in ferrets and healthy adults have confirmed these predictions, to date. Therefore, no special precautions will be needed for administration to participants who may have household members or other close contacts with immunosuppressive conditions.

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

Placebo:
The placebo is intranasally administered physiological saline, i.e., 0.9% sodium chloride for injection, which is given by spray in the nares. Side effects do not typically occur from administration of this solution to the nasal passages. If the participant's nasal passages are dry or irritated, it is possible he or she may experience a stinging sensation or have some mild rhinitis. It is possible that the administration itself could lead to mild bleeding in those with dry or edematous nasal mucosa.

Licensed IIV4:
Licensed, injectable inactivated seasonal influenza vaccine will be given to all participants for whom it is not contraindicated. It is not expected that the risks will be different in frequency or magnitude, when compared to those encountered among children receiving IIV4 as part of standard care and described below.

> Local and Systemic Reactions to IIV4: Recipients of unadjuvanted licensed IIVs may develop influenza-like reactions, such as fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), arthralgia (joint pain), headache, and/or nausea. Some persons may develop reactions at the injection site, including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and/or tenderness. Most of these reactions peak in intensity in the first 24 hours after vaccination and disappear without treatment within 1 or 2 days. Analgesics (e.g., acetaminophen or ibuprofen or similar non-steroidal anti-inflammatory drugs [NSAIDs]) and rest may generally relieve or lessen these reactions. Bruising can sometimes occur due to the vaccination procedure.
>
> Potentially Immune Mediated Medical Conditions (PIMMCs) Associated with IIV4: In post-marketing surveillance, potentially autoimmune, auto-inflammatory, and immune-mediated diseases have been reported after receipt of seasonal influenza vaccines. These disorders include neuritis, convulsions, severe allergic reactions, syncope, encephalitis,

thrombocytopenia, and vasculitis. [14] Reports of these reactions are rare and may or may not exceed the rates in unvaccinated persons; exact incidence rates cannot be precisely calculated. There has been no consistent reporting of individual immune-mediated diseases or the aggregate of all these diseases in persons receiving influenza vaccines or vaccines directed against any pathogen.

Guillain-Barré Syndrome (GBS) Associated with IIV4: During a 1976 vaccine campaign directed against swine influenza, which was caused by a type A H1N1 influenza virus, some recipients developed a paralytic illness called Guillain-Barré Syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza vaccine with a frequency of about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines.

Most persons who develop GBS recover completely. The recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack.

Intensive surveillance for GBS after administration of inactivated influenza vaccines since 1976 has shown a slight increase in risk over background cases (more than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination [6]. Interestingly, although vaccination rates have increased in the last 10 years, the numbers of reported cases of vaccine associated GBS have declined [7]. A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older [3]. An active, population-based surveillance study conducted in the United States during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines [9-11]. Another study, using the Medicare system, showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48) [12]. An international collaborative study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS [13].

It is unknown if the administration of the Sing2016 M2SR H3N2 vaccine and seasonal vaccines to be used in this study will result in an incidence of GBS that exceeds the baseline rate. GBS has not been associated with LAIV.

2.3.1.2 Potential Risks Associated with Drawing Blood or Receipt of an Intramuscular Injection or Intranasal Spray

Phlebotomy Risks:

Drawing blood is typically well tolerated and uneventful. It often causes transient mild discomfort. Approximately 2% of persons who have blood drawn will experience "near syncope", in which they feel lightheaded or have other symptoms, but do not lose consciousness. Syncope (fainting) is a transient autonomic (vasovagal) response to phlebotomy that occurs in approximately 1% of blood donors and fewer than 1% of those having diagnostic phlebotomy.[15] It is managed by having the participant lie down, loosening his or her clothes, monitoring vital signs, reassuring the participant, and giving fluids. Minor bruising at the blood draw site may occur in up to 12% and a hematoma in 2-3%. These events can be prevented or decreased in frequency by employing experienced phlebotomists and can be ameliorated, when they occur, by applying pressure to the draw site for several minutes. Rarely, following phlebotomy, persons may have nerve injury (typically lateral antebrachial nerve) causing pain, paresthesia, and motor or sensory loss. This phenomenon is uncommon and typically resolves quickly with watchful waiting only.[16] Infection at the site of the blood draw is exceedingly rare and can be avoided by using antiseptics and other appropriate techniques. In a study of over 4000 persons undergoing routine venipuncture for insurance applications, there were no serious local reactions such as cellulitis or phlebitis.[15]

Intramuscular (IM) Injection Risks:
The injection of a vaccine into the deltoid or anterolateral aspect of the upper thigh muscle, as will be done in this study when participants are given IIV4, is typically well tolerated. However, it often causes transient discomfort. Intramuscular injections may cause near syncope or syncope, although less commonly than phlebotomy. They can also cause an abscess, a hematoma, injury to blood vessels and peripheral nerves, or tingling or numbness. Injection too proximally on the lateral aspect of the deltoid can lead to injury of the bursa, which can cause Shoulder Injury Related to Vaccine Administration, or SIRVA.[17] Proper education and technique reduce the risk of adverse events after IM injection.

Intranasal Administration Risk:
Regardless of the product administered, giving an intranasal spray may lead to sneezing, nasal bleeding, dryness, pain/irritation, and edema.

2.3.1.3 Potential Risks Associated with Nasal Washes and Nasal Swabs

Obtaining a nasal wash (for collection of specimens for immunologic analysis) or the obtaining a nasal swab (for diagnosis of a respiratory pathogen) may cause mild discomfort and on rare occasions may cause a nosebleed. Nosebleeds due to a nasal wash are transient and can be managed by applying pressure for a short duration of time.

2.3.1.4 Potential Risks Associated with Pregnancy

It is unknown if this vaccine poses any risks to a pregnant woman or her unborn child. As such, female participants of childbearing potential, as defined in the Eligibility Criteria, must agree to use an effective method of birth control for at least 30 days prior to the study vaccination and for at least 60 days following the study vaccination. Males who are sexually active with a female of childbearing potential must agree not to father a child for 60 days after receipt of the study vaccination. Participants in all cohorts except Cohort 1 are too young (under age 9) to be of childbearing potential or to father a child.

2.3.1.5 Risk of Loss of Confidentiality

Parents/guardians of participants will be asked to provide the personal/protected health information (PHI) of the participating child. All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will gain access to this PHI. All hard copy study records will be kept in a locked file cabinet or maintained in a locked room at the participating site. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Any publications from this trial will not use information that will identify participants by name. Organizations that may inspect and/or copy research records maintained at the participating site for quality assurance and data analysis, include groups such as the local Institutional Review Board (IRB), NIAID, and the FDA.

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

2.3.1.6 Unknown Risks

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

2.3.2 Known Potential Benefits

Participants in this study may have no personal, direct benefits from participation. Even without any personal direct benefits, participants may contribute to a societal benefit through the improvement of our understanding of these vaccines. For those participants allocated to the vaccine groups, they may receive additional protection against influenza, beyond the protection afforded by receipt of seasonal influenza vaccine. All participants will be given routine licensed influenza vaccine (IIV4) and will be afforded the associated benefits. And each participant will have careful follow-up through the respiratory viral season that may provide some additional benefit.

2.3.3 Assessment of Potential Risks and Benefits

Individual participants take on the minor risks delineated in the protocol, which are necessary to provide the scientific information that could allow for use of this vaccine in the future, in children. Although data on the safety and immunogenicity of the vaccine in adults is informative, it does not substitute for data collected from studies that enroll children. The potential risks are minimized by providing study product prior to the availability of seasonal influenza vaccine, carefully designing the study with defined safety oversight measures, performing the study at sites with experience in studying influenza vaccines, and by having experienced study personnel perform study related procedures. The value of the information to be gained outweighs the risks of participation.

3. OBJECTIVES AND ENDPOINTS

Primary

1. To assess the safety and tolerability of one or two administrations of the Sing2016 M2SR H3N2 influenza vaccine at 10^8, or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

Secondary

2. To assess the humoral immunogenicity (serum antibody and mucosal antibody responses) directed against homologous viral strains after one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

Exploratory

3. To assess the cellular immunogenicity (T-cell immune responses) against Sing2016 M2SR H3N2 influenza vaccine following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, and 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
4. To assess participant immunological response to neurominidase (NA) following one and two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
5. To assess the humoral immunogenicity (serum and/or plasma antibody) and mucosal antibody responses, and cellular immunogenicity (T-cell responses) after seasonal influenza vaccine (IIV4) following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, and 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
6. To assess mucosal antibody responsesdirected against homologous viral strains after one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
7. To assess humoral immunogenicity (serum and/or plasma antibody), and mucosal antibody responses) directed against heterologous viral strains of one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8 or 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
8. To conduct additional characterization of humoral immunity against homologous and/or heterologous viral strains (e.g., extra-neutralizing antibody function) following one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, and 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age
9. To assess the effects of age, dose level, sex, prior receipt of seasonal influenza vaccine(s), and other variables on humoral immunogenicity (serum antibody and mucosal antibody responses) directed against homologous viral strains of one or two administrations of Sing2016 M2SR H3N2 influenza vaccine at 10^8, and 10^9 $TCID_{50}$ delivered intranasally to healthy participants, 2 to 17 years of age

<u>Study Endpoints</u>

Primary

1. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience solicited local reactogenicity events, of all severity grades and by grade, in the 7 days following administration of each dose of vaccine
2. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience solicited systemic reactogenicity events, of all severity grades and by grade, in the 7 days following administration of each dose of vaccine

3. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience unsolicited non-serious adverse events, of all severity grades and by grade, in the 28 days following administration of each dose of vaccine
4. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience adverse events of special interest (AESIs) the time of first vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)
5. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience Serious Adverse Events (SAEs) from the time of first vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)
6. The number and percentage of study participants in Cohorts 1-4 each and across Cohorts 1-4 who experience new-onset chronic medical conditions (NOCMCs) from the time of the first study vaccination through the end of the study period (final visit in the month of April of the calendar year following enrollment)

Secondary

7. The number and percentage of participants in Cohorts 1, 2, and 3 with putative seroprotection at baseline (Day 1) and on approximately Day 29, defined as an HAI titer ≥1:40 in serum against an H3N2 M2SR-like virus
8. The number and percentage of participants in Cohorts 4 with putative seroprotection at baseline (Day 1) and on approximately Day 57, defined as an HAI titer ≥1:40 in serum against an H3N2 M2SR-like virus
9. The number and percentage of participants in Cohorts 1, 2, 3 with neutralization titer ≥1:40 in serum against an H3N2 M2SR-like virus on approximately Day 29
10. The number and percentage of participants in Cohorts 4 with neutralization titer ≥1:40 in serum against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 57
11. Geometric Mean Titers (GMTs) of serum HAI against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29 for participants in Cohorts 1, 2, and 3
12. Geometric Mean Titers (GMTs) of serum HAI against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 57 for participants in Cohorts 4
13. Geometric Mean Titers (GMTs) of serum neutralizing antibodies against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1) and on approximately Day 29
14. Geometric Mean Titers (GMTs) in serum neutralizing antibodies against an H3N2 M2SR-like virus for participants in Cohorts 4at baseline (Day 1) and on approximately Day 57
15. Geometric Mean Fold Rise (GMFR) and proportions in serum with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in HAI titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
16. Geometric Mean Fold Rise (GMFR) and proportions in serum with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in HAI titers against an H3N2 M2SR-like virus for participants in Cohorts 4 on approximately Day 57

17. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in serum neutralization titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
18. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in serum neutralization titers against an H3N2 M2SR-like virus for participants in Cohorts 4 on approximately Day 57
19. Mean secretory IgA (sIgA) responses, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 before vaccination and on approximately Day 29
20. Mean secretory IgA (sIgA) responses, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 4 before vaccination and on approximately Day 57
21. Mean change (difference) from baseline in secretory IgA (sIgA) responses, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
22. Mean change (difference) from baseline in secretory IgA (sIgA) responses, as measured by the binding antibody multiplex assay (BAMA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 4 on approximately Day 57

Exploratory

23. Antigen-specific T-cell responses to vaccine immunogens, at baseline (Day 1) and following one or two doses of vaccine
24. Humoral antibody, as detected by one or more assays that measure responses to NA, at baseline (Day 1) and following one or two doses of vaccine or IIV4
25. Serum antibody, as detected by HAI and neutralization, directed against homologous H3N2 strains at baseline (Day 1) and after IIV4 administration
26. Antigen-specific T-cell responses to vaccine immunogens, at baseline (Day 1) and measured after IIV4 administration
27. Serum antibody to additional influenza antigens, as detected by multiplex ELISA assays and/or protein microarrays with subsequent evaluation of HAI and neutralization when multiplex ELISA screen is positive, directed against heterologous strains at baseline (Day 1) and following one or two doses of Sing2016 M2SR H3N2 vaccine
28. Humoral and/or mucosal antibody neutralizing and extra-neutralizing function against homologous and heterologous strains based on positive responses detected by methods described above.
29. Associations between serum antibody responses and age, dose level, sex, prior receipt of seasonal influenza vaccine(s) and other variables over time.
30. Associations between mucosal antibody responses and age, dose level, sex, prior receipt of seasonal influenza vaccine(s) and other variables over time.
31. Geometric Mean Titers (GMTs) of plasma HAI against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29 for participants in Cohorts 1, 2, and 3
32. Geometric Mean Titers (GMTs) of plasma HAI against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 57 for participants in Cohort 4
33. Geometric Mean Titers (GMTs) of plasma neutralizing antibodies against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1) and on approximately Day 29

34. Geometric Mean Titers (GMTs) of plasma neutralizing antibodies against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1) and on approximately Day 57
35. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma HAI titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
36. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma HAI titers against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
37. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma neutralization titers against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
38. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma neutralization titers against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
39. The number and percentage of participants in Cohorts 1, 2, and 3 with putative seroprotection at baseline (Day 1) and on approximately Day 29, defined as a plasma HAI titer ≥1:40 against an H3N2 M2SR-like virus
40. The number and percentage of participants in Cohort 4 with putative seroprotection at baseline (Day 1) and on approximately Day 57, defined as a plasma HAI titer ≥1:40 against an H3N2 M2SR-like virus
41. The number and percentage of participants in Cohorts 1, 2, 3 with plasma neutralization titer ≥1:40 against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 29
42. The number and percentage of participants in Cohort 4 with plasma neutralization titer ≥1:40 against an H3N2 M2SR-like virus at baseline (Day 1) and on approximately Day 57
43. Geometric mean titers (GMTs) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29
44. Geometric mean titers (GMTs) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
45. Geometric mean fold rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
46. Geometric mean fold rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in secretory IgA (sIgA) endpoint titers, as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
47. Mean total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for

participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29

48. Mean total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
49. Mean change (difference) from baseline in total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
50. Mean change (difference) from baseline in total secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
51. Mean normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 at baseline (Day 1, before vaccination) and on approximately Day 29
52. Mean normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens , against an H3N2 M2SR-like virus for participants in Cohort 4 at baseline (Day 1, before vaccination) and on approximately Day 57
53. Mean change (difference) from baseline in normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohorts 1, 2, and 3 on approximately Day 29
54. Mean change (difference) from baseline in normalized secretory IgA (sIgA), as measured by the enzyme-linked immunosorbent assay (ELISA) in nasal lavage specimens, against an H3N2 M2SR-like virus for participants in Cohort 4 on approximately Day 57
55. Geometric Mean Titers (GMTs) of plasma or serum NA antibodies against an H3N2 NA for participants in Cohorts 1, 2, and 3 at baseline (Day 1) and on approximately Day 29
56. Geometric Mean Titers (GMTs) of plasma or serum NA antibodies against an H3N2 NA for participants in Cohort 4 at baseline (Day 1) and on approximately Day 57
57. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma or serum NA antibodies against an H3N2 NA for participants in Cohorts 1, 2, and 3 on approximately Day 29
58. Geometric Mean Fold Rise (GMFR) and proportions with greater than or equal to 2- and 4-fold rises from baseline (Day 1) in plasma or serum NA antibodies against an H3N2 NA for participants in Cohort 4 on approximately Day 57
59. The number and percentage of participants in Cohorts 1, 2, 3 with NA antibody titer ≥1:40 against an H3N2 NA at baseline (Day 1) and on approximately Day 29
60. The number and percentage of participants in Cohort 4 with NA antibody titer ≥1:40 against an H3N2 NA-like virus at baseline (Day 1) and on approximately Day 57

4. STUDY DESIGN

4.1 Overall Design

This is a Phase 1b, randomized, double-blind, dose-escalating, age de-escalating, placebo-controlled study of 200 children 6 months through 17 years of age, inclusive, who are in good health and meet all eligibility criteria. To allow for simultaneous enrollment at the four research sites, the number of children in each cohort and the total number of children in the entire study may slightly deviate from the targeted numbers mentioned throughout the protocol.

The study design includes 7 cohorts in Protocol Versions 1-4. Given a number of non-safety-related contingencies (timelines, budgets, recruiting, and lack of additional vaccine lots), Cohorts 5, 6, and 7 will no longer be enrolled as of Protocol Version 5.0. Cohorts 5, 6, and 7 included children 6 to 23 months old. The study-related objectives and endpoints have been updated to reflect that only Cohorts 1 through 4, were enrolled. Descriptions of Cohorts 5, 6, and 7 have not been removed from the remainder of the protocol design.
All participants in Cohorts 1, 2, 3, and 4, will be "non-naïve", having been vaccinated against seasonal influenza in a prior season. Cohorts 5, 6, and 7 will be 6 to 23 month-olds who are influenza-naïve (no previous influenza vaccine and no previous documented influenza infection).

A cohort of 45 healthy children 9-17 years old (Cohort 1) will receive a single dose of vaccine at the 10^9 $TCID_{50}$ dose of the Sing2016 M2SR H3N2 vaccine or placebo. At the same time, the second cohort (Cohort 2) will begin enrollment.

Cohort 2 will consist of 45 healthy children 2-8 years old who will also receive a single dose of the vaccine, but they will be given at 10^8 $TCID_{50}$ or placebo. Once there is sufficient evidence of safety and tolerability in Cohorts 1 and 2 and enrollment has completed of all 45 in each of these cohorts, the third cohort (Cohort 3) will begin enrollment.

Cohort 3 will consist of 25 healthy children 2-8 years old who will receive a single dose of the 10^9 $TCID_{50}$ dose of vaccine or placebo. Once there is sufficient evidence of safety and tolerability in Cohort 3 and enrollment has completed for this cohort, the fourth and fifth cohorts (Cohorts 4 and 5) will begin enrollment.

Cohort 4 will consist of 25 healthy children 2-8 years old who will receive 2 doses of the 10^9 $TCID_{50}$ dose of vaccine or placebo. Participants in Cohort 4 will enroll concurrently with participants in Cohort 5.

Cohort 5 (termed lead-ins) will consist of 8 children, 6-23 months old, who will be randomized to receive two doses of 10^7 $TCID_{50}$ Sing2016 M2SR (n=6) or two doses of placebo (n=2).

Once there is sufficient evidence of safety and tolerability in Cohort 5 and enrollment has completed for Cohort 5, Cohort 6 will open for enrollment. Once 6 of the 8 participants in Cohort 5 have completed Day 4 after the second dose (e.g., Study Day 32), the following process will be implemented prior to receipt of the second dose for any participant in Cohort 6:

1. An email will be sent from the site to the SDMCC attesting that all data entry through Day 4 after the second dose (e.g., Study Day 32) for at least 6 participants in Cohort 5 has been completed.

2. The SDMCC will perform data cleaning as it pertains to assessment of the halting rules.
3. The SDMCC will assess if there were any halting rules met based on data entered in the data system and email DMID indicating what halting rules, if any, were met based on data entered in the data system.
    a. If any of the halting rules were met, the study will halt and a review by the SMC, sponsor, and investigators will convene as specified in Section 7.1.
    b. If none of the halting rules were met, DMID will confirm that the study may proceed as per-protocol.

Cohorts 6 will consist of a lead-in group of 8 children, 6-23 months old followed by an additional 18. The first 8 will be randomly assigned to receive either two doses of 10^8 $TCID_{50}$ of the Sing2016 M2SR (n=6) or two doses of placebo (n=2). Once the first 8 children have completed Day 8, the SRC will review the safety and determine if Cohort 7 may begin enrollment. Enrollment of the remaining 18 Cohort 6 participants may continue during SRC review and must be completed before enrollment into Cohort 7 can begin.

Cohort 7 will consist of 26 healthy children 6 to 23 months old, 18 of whom will be randomly assigned to receive 2 doses of the 10^9 $TCID_{50}$ dose of vaccine and 8 of whom will be randomly assigned to receive placebo.

This study is designed to assess the safety and immunogenicity of one or two doses of the Sing2016 M2SR H3N2 influenza vaccine delivered intranasally. Eligible participants will be enrolled and randomly assigned to receive the Sing2016 M2SR H3N2 vaccine (one or two doses) or placebo. An overview of the planned cohorts is presented in Table 2. All participants will be administered the IIV4 seasonal influenza vaccine a minimum of 28 days after last IP dose or later once it is available (anticipated September – November). IIV4 may be administered outside of the study window described in the protocol, in case of public health emergency or as determined necessary by institutional policy, regulatory authorities, sponsor or principal investigator. Management of participants who meet this criterion will be as determined suitable by site-specific procedures. COVID-19 vaccines which are fully approved or approved under EUA may be received during study participation. COVID-19 vaccines may not be received within the 10 days before receipt of the study product nor planned to be received during a reactogenicity period (the day of and 7 days following each intranasal vaccination) of the study product. Participants who experience a COVID-19 exposure during study participation will be managed as per institutional protocol.

Due to the limited availability of product, and funding to support additional years of enrollment into Cohorts 5, 6, and 7, the decision was made to stop enrollment after the final participant was enrolled into Cohort 4.

Table 2. Cohorts

Cohort	Age	Experience	Vaccine	Dose	Doses	Participants
1	9-17 yrs.	Non-naive	Sing2016 M2SR H3N2	10^9 $TCID_{50}$	1	30
			Placebo	-	1	15

2	2-8 yrs.	Non-naive	Sing2016 M2SR H3N2	10^8 $TCID_{50}$	1	30
			Placebo	-	1	15
3	2-8 yrs.	Non-naive	Sing2016 M2SR H3N2	10^9 $TCID_{50}$	1	15
			Placebo	-	1	10
4	2-8 yrs.	Non-naive	Sing2016 M2SR H3N2	10^9 $TCID_{50}$	2	15
			Placebo	-	2	10
5	6-23 mo.	Naïve	Sing2016 M2SR H3N2	10^7 $TCID_{50}$	2	6
			Placebo		2	2
6	6-23 mo.	Naïve	Sing2016 M2SR H3N2	10^8 $TCID_{50}$	2	18
			Placebo	-	2	8
7	6-23 mo.	Naïve	Sing2016 M2SR H3N2	10^9 $TCID_{50}$	2	18
			Placebo	-	2	8
Total Sample size						200

Schedule of assessments can be found in **Section 1.2, Schedule of Activities.**
Protocol Schema can be found in **Section 1.3, Protocol Schema**.
Dose escalation or dose-ranging details are found in **Section 6.1.2, Dosing and Administration**
Full details of interim analysis are found in **Section 9.4.6, Planned Interim Analyses**

4.2 Scientific Rationale for Study Design

Currently available inactivated and recombinant influenza vaccines induce neutralizing antibodies that recognize the virus envelope protein HA. These antibodies effectively induce immunity, but the efficacy of these vaccines depends 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 original vaccine viral strain. About 90% of vaccines of the currently available inactivated vaccines have limited or no broadly cross-reactive (heterosubtypic) immunity to protect against divergent or drifted strains of the virus. Currently available trivalent (IIV3) or quadrivalent (IIV4) preparations provide the HA antigen and either none or limited and non-standardized amounts of the NA antigen. Moreover, at least one HA component of these vaccines must often be updated each influenza season to account for accumulated point mutations in the HA protein that allow the viruses to evade circulating human immune response. In addition, while a strong relationship has been established between pre-existing T-cell immunity and illness severity in persons seronegative for influenza virus-specific antibody, no or minimal cellular immune responses have been shown to be elicited following vaccination with these conventional preparations [3].

Although live influenza virus vaccines may offer immunologically superior responses because they induce diverse types of adaptive responses, only one such product, FluMist®, has been approved in the US, and is presently indicated only for certain persons 2-49 years of age [18]. Accumulating data with FluMist® also indicate that pre-existing, cross-reactive immunity, present in most adults, limits vaccine virus replication, which in turn mitigates a consistently effective immune response.

In an effort to overcome the limitations of currently available influenza vaccines, FluGen, Inc. is developing a novel live influenza virus vaccine platform known as "M2SR". This platform is based on a replication-defective recombinant influenza virus that does not express M2 protein (and hence, the derivation of the "M2SR" naming convention [*M2* deficient *S*ingle *R*eplication vaccine]). The M2SR vaccine virus is comprised of the following: (1) 5 of the 8 gene segments of the donor virus, Influenza A/Puerto Rico/8/34 (PR8), an attenuated strain that has been used for decades in traditional inactivated influenza vaccine manufacturing; (2) two genetic segments for influenza virus surface protein antigens, HA and NA, derived from any selected type A influenza strain; and (3) an M gene segment that cannot express the M2 protein. In the vaccine virus, the M2 protein is acquired from the cell substrate used to grow the vaccine, M2 Vero cells, which stably expresses and supplies M2 protein for vaccine virus growth. The resulting vaccine virus can infect normal cells in the respiratory epithelium of the vaccine recipient and then uncoat and initiate infection in a manner similar to a wild-type influenza virus, thereby evoking an immune response. However, because the M2SR genome does not encode for the M2 protein, no viral progeny are produced after the initial (single-round) infection, such that no cell-to-cell spread, or subsequent shedding of virus occurs.

The HA and NA of an influenza A/Brisbane/10/2007-like H3N2 virus were chosen for inclusion in the prototype monovalent vaccine that was initially tested in humans, and this prototype vaccine is referred to here as "Bris10 M2SR H3N2 vaccine". A second investigational (updated) H3N2 M2SR vaccine encodes the HA and NA of the A/Singapore/INFIMH-16-0019/2016 reference virus, the H3N2 strain that was recommended for the 2018-2019 influenza season and is referred to here as "Sing2016 M2SR H3N2 vaccine". Sing2016 M2SR H3N2 vaccine contains a similar PR8 backbone to Bris10 M2SR H3N2 vaccine 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. This updated vaccine of the M2SR platform was generated in a similar manner as Bris10 M2SR H3N2 vaccine. Sing2016 M2SR H3N2 vaccine is the vaccine that will be evaluated in this study.

4.3 Justification for Dose

Previous clinical experience with the 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, an on-going Phase 1b dose escalation study in healthy adults, and an on-going Phase 1 safety and immunogenicity study in 9-17 years, sponsored by NIAID/DMID (DMID 17-0012, NCT03553940 – preliminary results posted). A total of 410 adults and 43 children, respectively, have been enrolled in these studies to date.

In the FTIH study, 72 adults (24 participants per dose level) received a single dose of Bris10 M2SR H3N2 vaccine at levels of 10^6, 10^7, or 10^8 $TCID_{50}$. In the challenge study, 52 adults

received a single dose of Bris10 M2SR H3N2 vaccine, with 48 subsequently challenged with a drifted H3N2 strain. The M2SR influenza vaccines evaluated to date have not displayed any evidence of shedding in human participants (n=285) evaluated in the FTIH, Phase 2a and Phase 1b studies. These results align with the biology of the M2SR virus; that is, the replication-deficient phenotype is intrinsic to the virus (not based on host response) due to the deletion of the essential M2 gene.

In a Phase 1 study of 9-17 year-olds, 43 participants (out of 50 planned) were enrolled and received one 10^8 $TCID_{50}$ dose of Bris10 M2SR H3N2 vaccine or placebo, followed by one dose of licensed quadrivalent influenza vaccine (IIV4) administered 3 months later; data from this study remain blinded as of March 2021. Although the allocation to vaccine or placebo is not yet unblinded, among participants who received one dose of 10^8 $TCID_{50}$ M2SR vaccine, there have been no reported SAEs and no halting rules met.

For the Phase 1b dose escalation study, 206 healthy male and non-pregnant female participants ages 18-49 years old, having microneutralization (MN) titers of ≤ 20 to H3N2 A/Singapore/INFIMH-16-0019/2016, were randomized 1:1:1:1:1 to receive two administrations 28 days apart of Sing2016 M2SR H3N2 vaccine at three dose levels, Bris10 M2SR H3N2 vaccine at one dose level or placebo. Treatment groups included Sing2016 M2SR H3N2 vaccine at 10^8 $TCID_{50}$ (n=38), $10^{8.5}$ $TCID_{50}$ (n=35), and 10^9 $TCID_{50}$ (n=30); Bris10 M2SR H3N2 vaccine at 10^8 $TCID_{50}$ (n=39); and placebo (n=35). The first participant first dose was in September 2019 and the last participant completed the Day 209 follow-up visit (180 days following the second dose) in June 2020. The active phase of this Phase 1b dose escalation study was completed with no SAEs or observations that would halt the study.

Participants who received Bris10 M2SR H3N2 vaccine (10^8 $TCID_{50}$ dose) in the Phase 1a and Phase 2a studies 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 belong to the same clade (3c.3b) as the challenge virus used in the Phase 2a study. In the Phase 2a study, data demonstrate that the vaccine was effective in prevention of infection after challenge with an antigenically drifted virus, but mainly among participants for whom a humoral immune response to the vaccine was detected. Participants who demonstrated a 2-fold increase from baseline in serum antibody response (~50% of the M2SR vaccine cohort) by MN to the intranasal M2SR vaccine had lower viral load and influenza-like symptoms than placebo recipients. These preliminary results suggest that M2SR could potentially provide higher efficacy levels if a greater proportion of M2SR vaccine recipients can demonstrate an appropriate serological response.

In the subsequent Phase 1b study, a single 10^9 $TCID_{50}$ dose of Sing2016 M2SR H3N2 vaccine generated significantly increased serum HAI responses compared to the 10^8 $TCID_{50}$ of Bris10 M2SR H3N2 vaccine that had provided protection against infection and illness in the earlier human influenza challenge study. HAI titers ≥40 were achieved in 0%, 23% and 58% of participants after the first dose of placebo, 10^8, or 10^9 $TCID_{50}$ M2SR vaccine, respectively ($p<0.003$). Increases also were stimulated in serum MN titers to drifted strains of H3N2 and in serum NA inhibition (NAI) and mucosal sIgA titers. Further increases in serum and mucosal immune response were noted after a second IN vaccination.

The purpose of this proposed Phase 1b dose-finding clinical study is to assess the safety, tolerability/reactogenicity, and immunogenicity of the Sing2016 M2SR H3N2 vaccine, including doses from 10^8 $TCID_{50}$ to 10^9 $TCID_{50}$, in a pediatric population. The same Sing2016 M2SR H3N2 vaccine which will be used in this study is currently being investigated in adults, as described above. Demonstration of broadly reactive responses in adolescents and children, like those found to be protective against challenge in adults, would support further clinical development of the M2SR vaccine platform in this age group.

5. STUDY POPULATION

5.1 Inclusion Criteria

1. Participant is a male or female child aged 6 months to 17 years inclusive at time of enrollment (each cohort has its own age upper and lower limits[2])
   [1]Cohort 1: 9-17 years (on or after the 9th birthday and before the 18th birthday at the time of the first dose); Cohorts 2, 3, and 4: 2-8 years (on or after the 2nd birthday and before the 9th birthday at the time of the first dose); Cohorts 5, 6, and 7: 6 months to 23 months (on or after the 6th month of life based on calendar day and before the second birthday at the time of the first dose)
2. For Cohorts 1 to 4, receipt of at least 2 doses of seasonal influenza vaccine in the past.
3. For Cohorts 5 to 7, receipt of no seasonal influenza vaccines in the past and no documented history of laboratory-confirmed influenza illness
4. Parent/guardian of the participating child provides written informed permission and participating child provides assent[2] prior to initiation of any study procedures
   [2]as appropriate by age or development and approved by the IRB
5. Parent/guardian and participant, as appropriate, are able to understand and comply with planned study procedures and are available for all study visits
6. Participant is in good health as assessed by the principal investigator or other designated study investigator[3]
   [3]based on medical history and physical examination (physical examination may be done as part of routine medical care or specifically for eligibility screening)
7. Parent/guardian of the participating child agrees not to allow the participant to join another clinical trial that includes an investigational agent or device during the study period
8. A female participant of child-bearing potential[4] agrees to abstain from sexual intercourse or to correctly use an acceptable method of contraception[5]
   [4]A female of child-bearing potential is defined as a female who is post-menarchal and not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure. This applies only to participants in Cohort 1.
   [5]Acceptable methods of contraception must be used from 30 days prior to vaccination until 60 days after the last study vaccination (not IIV4) and include full abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more or shown to be azoospermic prior to the participant receiving the study vaccination, barrier methods such as condoms or diaphragms/cervical cap, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives ("the pill").

9. A female participant of child-bearing potential[4] must have a negative urine pregnancy test within 24 hours prior to each study product
10. A male who is sexually active with a female of childbearing potential[4] must agree to use an acceptable method of contraception[6]

[6]From the time of the first dose of study vaccine until 60 days after receipt of the last dose study vaccine, only in Cohort 1. The only acceptable method of contraception for males who are sexually active with females of childbearing potential is condoms.

5.2 Exclusion Criteria

1. Has a body temperature of 38.0°C/100.4°F (oral or axillary) or greater or another acute illness[7] within the 72 hours prior to study vaccination

[7]Potential participants who are recovering from an acute illness and have residual minimal symptoms, which, in the opinion of the site principal investigator or appropriate sub-investigator, will not likely affect the evaluation of outcome measures are not ineligible. Temperature evaluation will not be performed as a study procedure on participants prior to administration of seasonal influenza vaccine

2. Has any medical or mental health disease or condition[8] that would render study participation unsafe, or would interfere with the evaluation of the responses

[8]in the opinion of the site principal investigator or appropriate sub-investigator

3. Has a history of provider-diagnosed asthma requiring the use of medications at any age, or has had a wheezing episode or use of medications to treat asthma in the 12 months prior to screening.
4. Has immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy
5. Has a diagnosis of or history of malignant neoplastic disease
6. Has taken oral, parenteral (intramuscular or intravenous), inhaled, or nasal corticosteroids of any dose within 30 days prior to study vaccination
7. Has known HIV, hepatitis B, or hepatitis C infection
8. Has known hypersensitivity or allergy to any components of the study vaccine or material in the nasal delivery device[9]

[9]Vaccine components: sucrose, sodium chloride, phosphate, glutamate; delivery device material: polycarbonate, polypropylene, synthetic rubber

9. Has a history of severe reactions following previous immunization with licensed or unlicensed influenza vaccines
10. Has a history of an anatomic disorder of the nares or nasopharynx
11. Has a history of chronic sinus infections
12. Has a history of or currently smokes or vapes
13. Has a history of Guillain-Barré syndrome
14. Use of aspirin- or salicylate-containing products in the 30 days prior to or intends to use these products in the 30 days following administration of the investigational vaccine
15. Has a history of documented influenza or receipt of influenza antiviral treatment in the 4 months prior to the first vaccination
16. Receipt of any antiviral drug within the week prior to or following the investigational vaccine.
17. Receipt of a licensed live vaccine within 30 days prior to the first study vaccination or plans to receive a licensed live vaccine within the 30 days after the last study vaccination.
18. Receipt of licensed inactivated non-influenza vaccine[10] within 14 days prior to the 1st study vaccination, or plans to receive licensed, inactivated vaccine within the 30 days after the last study vaccination

[10] Participants will be asked to avoid receipt of any routine licensed vaccines or vaccines under emergency use authorization during the periods described.

19. Receipt of an influenza vaccine within the 4 months prior to the first study vaccination or plans to receive an influenza vaccine following the last study vaccination. Seasonal IIV4 will be received by participants as part of this trial.
20. Receipt of immunoglobulin or other blood products within the 6 months prior to the first study vaccination or plans to receive during the period of study participation.
21. Receipt of an experimental[11] agent or device within the 6 months prior to the first study vaccination or expects to receive an experimental agent or device during the study period

    [11]Products for treatment or prevention of COVID-19, when received under Emergency Use Authorization [EUA] or full FDA approval and not as part of a clinical trial, will not be deemed "experimental" for the purposes of this criterion and will not make an otherwise eligible prospective participant ineligible.
22. Is a family member of study personnel or personnel directly involved in the conduct or monitoring of the study
23. Receipt of an approved or experimental product for treatment or prevention of COVID-19 within the 10 days[12,13] prior to study enrollment

    [12]Participants may enroll if greater than 10 days after receipt of the COVID-19 treatment or prevention.
    [13]Participants who are receiving COVID-19 vaccines around the time of dosing of the investigational product will be asked to avoid COVID-19 vaccination within the 10 days before any vaccination in the study and within any reactogenicity period (the day of and 7 days following each intranasal vaccination).
24. Inability of the study team to collect 5 mL of blood from the participant before the first vaccination (pre-vaccination blood).

5.2.1 Exclusion of Specific Populations

This study is evaluating the immunogenicity of the Sing2016 M2SR H3N2 vaccine in pediatric participants. This evaluation is limited to children and hence the study will exclude adult and geriatric populations.

5.3 Inclusion of Vulnerable Participants

This study will provide clinical research data to inform influenza vaccine safety and immunogenicity in children. The children who take part in this study are considered vulnerable participants per the US Code of Federal Regulations, and site IRBs/IBCs will consider the potential risks and benefits to child participants as described in 45 CFR 46 and 21 CFR 50 Subparts D (for children).

With respect to these regulations, IRBs/IBCs will determine the level of risk to children in the categories specified. Documentation of this determination is required, and the risk category assigned by the IRB/IBC further determines the parental informed consent requirements for the study at each site.

5.4 Lifestyle Considerations

No lifestyle restrictions will be expected of participants.

5.5 Screen Failures

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

randomization. Screen failures will be recorded on the screen failure log together with the reason for failure.

5.6 Strategies for Recruitment and Retention

5.6.1 Recruitment

Sites may use a variety of recruitment approaches, that may include but are not limited to direct recruitment at clinics, referrals from other providers, registries, electronic health record (EHR) messages, and use of online and social networking websites and applications. Recruitment materials will educate parents/guardians and, where age appropriate, participants, on the details of the clinical trial and the previously known safety information.

5.6.2 Retention

Once a child has received study product, study staff will make every effort to retain him or her in follow-up for the protocol-specified duration of follow-up, i.e., through the influenza season as defined in the protocol, thereby minimizing potential biases associated with loss to follow-up.

5.6.3 Compensation Plan for Participants

Compensation will be provided to the participant's parent/guardian based on each site's standard. The amount and means of disbursing will be reviewed and approved by each site's IRB, or the central IRB, if applicable.

5.6.4 Costs

There are no costs for the research tests, procedures, and study product while taking part in this trial. The investigational product and the seasonal influenza vaccine will be provided free of charge. Procedures and treatment for clinical care may be billed to the participant, participant's insurance, or third party.

6. STUDY PRODUCT

6.1 Study Product(s) and Administration

6.1.1 Study Product Description

6.1.1.1 Product 1: Sing2016 M2SR H3N2 Intranasal Influenza Vaccine

The Sing2016 M2SR vaccine is provided as a frozen liquid formulation in 2 mL cryovials. Each vial contains ~0.6 mL of vaccine formulated to contain infectious viral particles of Sing2016 M2SR encoding the HA and NA of influenza virus strain A/Singapore/INFIMH-16-0019/2016 (H3N2). The virus is suspended in Sucrose Phosphate Glutamate with Sodium Chloride (SPG-NaCl) buffer comprised of 10% 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 Sing2016 M2SR vaccine is clear to opalescent, colorless to slightly yellow suspension.

6.1.1.2 Product 2: SPG buffer to be used as the diluent for Product 1

The vaccine diluent, SPG-NaCl buffer (10% 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), is provided as a frozen, liquid formulation. It is a clear, colorless solution that is used to prepare dilutions of the investigational vaccine for the lower dose treatments.

6.1.1.3 Product 3: Placebo

The placebo consists of a commercially prepared 0.9% sodium chloride for injection, USP.

6.1.1.4 Product 4: Seasonal Inactivated Influenza Vaccine (IIV4)

All participants without contraindications will be given injectable IIV4 using a licensed product. All available IIV products licensed for children in the US are now quadrivalent.

6.1.1.5 Product 5: MAD300™ Sprayer Device

The intranasal delivery device, i.e., nasal spray device, is comprised of a MAD300™ mucosal atomization device and a 1 mL Henke-Ject (low dead space) syringe. The MAD300™ is made from radiation-stable medical-grade polycarbonate material and is compliant with USP Class VI and ISO 10993 requirements. It is manufactured by Teleflex Medical and is a conical shaped component with a Luer-lock feature for attachment to the filled syringe. For intranasal administration of the Sing2016 M2SR vaccine and placebo, the MAD300™ conical shape forms a plug in the nostril and rapid depression of the syringe plunger atomizes the liquid as it passes through the device nozzle into a fine mist.

6.1.2 Dosing and Administration

Participants will receive one of the following: the 10^9 $TCID_{50}$ dose or the 10^8 $TCID_{50}$ dose or the lowest dose of 10^7 $TCID_{50}$, of the Sing2016 M2SR H3N2 vaccine or placebo. Due to the limited availability of product and funding to support additional years of enrollment into Cohorts 5, 6, and 7, the decision was made to stop enrollment after the final participant was enrolled into Cohort 4. This means no participant will receive the 107 TCID50 dose.

The Sing2016 M2SR H3N2 vaccine will be provided to the site as a frozen, liquid formulation filled at a volume of 0.5 mL in 2.0 mL cryovials that are stored at ≤ -65°C. It is a clear to opalescent, colorless to yellow solution. Once thawed for use, the M2SR must not be refrozen for later use.

A pharmacist or his/her designee will thaw the vial contents to room temperature soon before dose administration, as described in the Manual of Procedures (MOP). The contents will be diluted to the target dosing concentration with provided Sucrose-Phosphate-Glutamate-NaCl (SPG-NaCl) diluent for each participant. The SPG-NaCl buffer is provided as a frozen, liquid formulation and in single use vials that are stored at ≤ -65°C. It is a clear, colorless solution. Once thawed for use, the SPG-NaCl must not be refrozen for later use.

The final diluted product will be drawn into two 1 mL disposable polypropylene syringes (Henke-Ject) and then each syringe will be fitted with a MAD300™ nasal device (MAD300*; Teleflex, Morrisville, NC, USA)* for intranasal delivery, using one intranasal delivery device (a syringe fitted with a nasal device) per naris.

Detailed instructions on preparation, concealing the syringe, timing from thaw to preparation to administration, and further study product details are found in the MOP.

Placebo control will be given as the comparator to the Sing2016 M2SR H3N2 vaccine. The saline solution placebo will be administered on the same schedule as the Sing2016 M2SR H3N2 vaccine product (Table 3) and will also be administered using the MAD300™ nasal device.

Seasonal IIV4 is given intramuscularly at the dose and volume recommended by the manufacturer for the age of the participant.

Table 3: Age and Dose Cohorts

Cohort	Age	Experience	Product Dose & Name	Route	Frequency
1	9-17y	Non-naive	10^9 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Single dose, D1
2	2-8y	Non-naive	10^8 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Single dose, D1
3	2-8y	Non-naive	10^9 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Single dose, D1
4	2-8y	Non-naive	10^9 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Two doses: D1, D29
5	6-23m	Naive	10^7 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Two doses: D1, D29
6	6-23m	Naive	10^8 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Two doses: D1, D29
7	6-23m	Naive	10^9 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo	IN	Two doses: D1, D29

6.1.3 Dose Escalation

In this study, Cohort 1 will first be administered 10^9 $TCID_{50}$ Sing2016 M2SR H3N2 or placebo, and Cohort 2 will be administered 10^8 $TCID_{50}$ Sing2016 M2SR H3N2 vaccine or placebo intranasally. Subsequent cohorts will be permitted to begin enrollment when criteria, as described in the protocol, have been met.

6.1.4 Dose Modifications

If adverse events are deemed to be occurring at frequencies or with severities that were unexpected, the sponsor, investigators, and SMC may take one of a number of actions. These include halting the study and adjusting the dose given to subsequent participants or cohorts. No changes will be implemented without IRB approval.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

The study product (vaccine and diluent) will be provided by FluGen, Inc. The seasonal IIV4, MAD300™ Sprayer Device, and saline will be sourced as specified by the MOP or advised by the study sponsor, which maybe locally or by DMID Clinical Materials Services (CMS). The

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

Product 1: Sing2016 M2SR H3N2 Intranasal Influenza Vaccine
Product 2: SPG-NaCl buffer to be used as the diluent for Product 1
Product 3: Saline
Product 4: Seasonal IIV4
Product 5: MAD300™ Sprayer Device

Upon request by DMID, study products 1, 2, 3, 4 and 5 will be transferred to the following address:

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

Based on availability, DMID will advise on sourcing of additional specific products which may include CMS or site-specific procurement. This includes saline placebo, IIV4, cryovials, Henke-Ject syringes and shipping supplies for this study. The study products will be provided through the DMID CMS to the participating clinical sites prior to the start of this trial upon request and with prior approval from DMID. Should the site PI require additional study products, further instructions will be provided in the protocol-specific MOP.

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

Used and unused of study products will be retained until study conclusion or until accountability via verification of inventory or monitoring has occurred and written notification stating retention is no longer required is received as applicable per the site's SOPs.

Accountability may be performed through verification of inventory and during monitoring. DMID does not require used containers of study product to be maintained at the research pharmacy until the clinical monitors have confirmed the disposition of all study products. Retention of used study product containers for monitoring is only required when the local institution's SOP/policy mandates it. If local SOPs allow/require destruction of used study

product containers, then the used CTM vials can be destroyed per the site's SOPs with a second staff member's observation and signed verification (two signatures) that the used CTM vials were destroyed.

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

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

6.2.2 Formulation, Appearance, Packaging, and Labeling

6.2.2.1 Product 1: Sing2016 M2SR H3N2 Intranasal Influenza Vaccine

The vaccine is manufactured in M2VeroA cells that provide the internal protein M2 for vaccine virus production. Cells are infected with the master virus seed and the infected cell culture supernatant is harvested at the peak of infection. The resulting virus-infected medium is then processed during downstream events to purify live virus from cell debris and production reagents. Steps are taken during purification to remove Vero cell DNA and host cell proteins and contaminants. The purified virus is formulated into the SPG formulation buffer that helps to stabilize the live virus. Instructions on sterile filtration and preparation of the drug product can be found in the MOP.

The Sing2016 M2SR H3N2 vaccine will be provided frozen and in cryovials. An unblinded pharmacist or their designee will thaw the vial contents to room temperature prior to dose administration, as detailed in the MOP. For all dose cohorts, the contents will be diluted if necessary, to the target dosing concentration with provided SPG-NaCl diluent for each participant.

The investigational product (whether undiluted [neat] or diluted) will be drawn into two intranasal delivery devices per participant dose. The plunger of each delivery device will be compressed to spray atomized solution into each nostril at administration.

6.2.2.2 Product 2: SPG-NaCl Buffer

The SPG-NaCl buffer is provided. It is clear, colorless solution. It is used as the diluent for the intranasal vaccine, Product 1. Complete instructions on vaccine preparation are found in the protocol-specific MOP.

6.2.2.3 Product 3: Saline placebo

The placebo consists of 0.9% sodium chloride for injection, USP (containing no preservatives and suitable for intranasal administration) and is a clear, colorless solution. It will be sourced by DMID CMS in single-use containers. The unblinded pharmacist or designee will aseptically withdraw volumes of the placebo as needed to fill the intranasal delivery devices.

6.2.2.4 Product 4: Seasonal IIV4

The licensed seasonal influenza vaccine will be acquired by DMID CMS with storage and administration as per manufacturers recommendations.

6.2.2.5 Product 5: MAD300™ Nasal Device

MAD300™ nasal devices are supplied in individual non-sterile pouches and are attached to sterile 1-mL single-use syringes before use (see Section 6.1.1.5). MAD300™ pouches will be stored as specified in the MOP.

Refer also to the MAD300™ Instructions for Use (IFU) as well as to the protocol-specific MOP.

Each of these study products, except the MAD300™ nasal device, will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use."

6.2.3 Product Storage and Stability

6.2.3.1 Product 1: Sing2016 M2SR H3N2 Intranasal Influenza Vaccine

Long term storage is at ≤ -65°C. Stability studies performed by FluGen demonstrate that the thawed vial contents remain functionally stable when diluted, if necessary, with SPG buffer to all doses required for the protocol as defined in the MOP.

6.2.3.2 Product 2: SPG-NaCl Diluent

SPG-NaCl buffer is stored frozen at ≤ -65°C. Stability studies indicate that the thawed vial contents remain stable for at least 48 hours when filled into the delivery device and held at 2-8ºC.

6.2.3.3 Product 3: Saline placebo

Stored at 20°C to 25°C (68°F to 77°F) (See USP Controlled Room Temperature). Placebo consists of a commercially prepared, physiological saline for injection, USP. Placebo, saline solution, is stable at room temperature.

6.2.3.4 Product 4: Licensed Seasonal IIV

Licensed seasonal IIV4 will be stored as per manufacturers recommendations.

The temperature of the storage units for study product will be recorded daily (excluding non-business days and holidays, as applicable), continuously monitored, and recorded during the duration of this trial per the participating CIVIC site's standard operating procedures, and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range.

In the event the temperature fluctuates outside of the required range, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at

DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturers will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine of product are provided in the protocol-specific MOP.

6.2.4 Preparation

See the protocol-specific MOP appendices for detailed information on the preparation, labeling, storage and administration of study vaccine for each group. It is recommended that the study vaccine preparation be performed by the participating CIVIC site's pharmacist on the same day of study vaccine administration. However, for logistical purposes, it is possible to prepare study vaccine prior to the day of dosing as long as dose administration occurs within 48 hours of vaccine stock removal from ultracold storage.

For each participant, a vial or vials of the study product and SPG-NaCl diluent as needed will be thawed and doses prepared as per the MOP.

The vaccine will be delivered intranasally (up to 0.3 mL per nostril as described in the MOP) using the intranasal delivery device. MAD nasal sprayer device employs a cone-shaped nozzle with an atomizer in the 4.3 mm tip and is attached to a 1 mL polypropylene syringe via a Luer lock feature. A MAD nasal sprayer device was previously successfully utilized for the intranasal delivery of live, wild-type A/H1N1 influenza virus in a human challenge study conducted as a Phase 1 study under US IND #BB-14969 by the National Institute of Allergy and Infectious Diseases, NIH (Memoli 2015; intranasal delivery of live, A/H1N1 influenza virus in a human challenge study under US IND 19213 (DMID protocol 18-0010); and a Phase 1 study under US IND # 18170 for intranasal delivery of H3N2 M2SR (DMID protocol 17-0012) [19]. The prepared dose will be drawn into two intranasal delivery devices that will be used to spray the contents into the participant's nostrils. Each nostril will receive approximately half of the targeted dose.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Treatment Assignment Procedures

Per ICH E6 GCP, screening records will be kept at the participating CIVIC site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Management Coordinating Center's (SDMCC) Advantage eClinical^{SM} (Electronic Data Capture System). Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the participant will be enrolled in the electronic data capture system. The participant will be enrolled and randomly assigned to either treatment or placebo control.

Enrollment of participants will be done online using the enrollment module of Advantage eClinical^{SM}. The randomization code will be prepared by statisticians at the SDMCC and included in the enrollment module for this trial. Advantage eClinical^{SM} will assign each participant to a treatment arm after the demographic and eligibility data have been entered into

the system. A designated individual at the CIVIC site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the Advantage eClinical[SM] User's Guide. Manual back-up procedures and instructions are provided for use in the event that the participating CIVIC site temporarily loses access to the internet or the online enrollment system is unavailable.

6.3.2 Randomization and Blinding

This is a randomized double-blinded study.

6.3.3 Blinding and Masking Procedures

This is a double-blind (masked) clinical trial. Participants, site investigators, and study personnel performing any study-related assessments following study product administration are blinded to product received. Laboratory personnel performing immunological assays will receive serum blinded to participant ID number, specimen visit number, and allocation group.

The randomization scheme will be generated by the SDMCC and provided to unblinded study personnel (i.e., research pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the participating CIVIC sites.

The unblinded or blinded study vaccine administrator is a study personnel member credentialed to administer vaccines. The unblinded vaccinator may also participate in dose preparation but will not be involved in study-related assessments or have participant contact for data collection following study vaccine administration. When participants receive IIV4, it is not necessary that vaccine is given by an unblinded member of the study team.

The SRC and the SMC will receive data in aggregate and the SMC may be presented data by aliased treatment group. The actual treatment assignments will be provided to the SMC at their request, but the SRC, as part of their review, will remain blinded. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual participant if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only.

6.4 Study Intervention Compliance

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

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and supplements.

Administration of any medications, therapies, or vaccines will be recorded on the appropriate eCRF. Concomitant medications will include all current medications and medications taken in the 30 days prior to enrollment. Study team will record, on the source document, medications taken starting from 30 days prior to the screening visit (or enrollment visit, if only one visit for screening and enrollment), but only enter into the eCRF, those medications taken up to 30 days prior to the enrollment. At subsequent visits, until Visit 4 (Day 29) for Cohorts 1, 2, and 3 (those receiving 1 dose of IN study product) and until Visit 7 (Day 57) for Cohorts 4, 5, 6, and 7 (those receiving 2 doses of IN study product) updates to previous concomitant medications and additional medications taken in the interim will be recorded. No concomitant medications will be recorded after Day 57. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. Use of a new medication should prompt evaluation for the occurrence of any AE, AESI, or SAE, if within the respective reporting period.

Other than those that preclude enrollment, as found in the eligibility criteria, medications that might interfere with the evaluation of the investigational product(s) should not be used from Day 1 until the time when, for the participant's cohort, concomitant medications are no longer being recorded, unless clinically indicated as part of the participant's health care. Use of antipyretics will not be encouraged. Parents may administer them to treat, but not to prevent, fever.

Medications in this category include the prohibited medications per the Participant Exclusion Criteria (see Section 5.2 Exclusion Criteria). Site team members will discourage prophylactic antipyretics but will encourage antipyretics to treat fever or other symptoms that may be relieved with these medications. In addition, the site PI or appropriate sub-investigator may identify other medications, herbals, vitamins, or supplements that should not be used due to a risk to participant safety or assessment of reactogenicity and immunogenicity.

6.5.1 Rescue Medicine

No rescue medications are applicable in this trial.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Halting Criteria and Discontinuation of Study Intervention

7.1.1 Study Halting Criteria Study-Wide

These halting criteria refer to all enrolled participants, study-wide, in aggregate and are cumulative. Should one occur, study enrollment will be paused for review by the SMC, sponsor, and investigators:

1. Any participant experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product if deemed related to the study product by the study investigator. These events must be reported to the medical monitor within 24 hrs of investigator awareness.
2. Two or more participants experience generalized urticaria (defined as occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product

3. Any participant experiences an SAE after administration of study product that is considered related to study product
4. Any participant experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product
5. 10% of participants (minimum 3 participants) experience the same grade 3 local solicited adverse event or systemic solicited adverse event (reactogenicity events, as defined in the protocol)
   10% of participants (minimum 3 participants) experience the same grade 3 unsolicited AE, in the same HLT by MedDRA coding, considered related to study product

7.1.2 Study Halting Criteria for "Lead-In" Participants

The 8 participants in Cohort 5 and the first 8 participants in Cohort 6 are termed "lead-ins" because data on safety and tolerability from Days 1 to 8 from these participants will allow for the following cohort to begin enrollment. Given that the lead-ins are young (6-23 months), influenza-naïve, and only 8 participants in Cohorts 5 and 6, the halting criteria are slightly more stringent. If any of the following events occurs, study enrollment will be paused for review by the SMC, sponsor, and investigators:

Any participant experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product if deemed related to the study product by study investigator. These events must be reported to the medical monitor within 24 hrs of investigator awareness.

1. Two or more participants experience generalized urticaria (defined as occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product
2. Any participant experiences an SAE after administration of study product that is considered related to study product
3. Any participant experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product
4. Two or more participants experience the same grade 3 local solicited adverse event or systemic solicited adverse event (reactogenicity events, as defined in the protocol)
5. Two or more participants experience the same grade 3 unsolicited AE, in the same HLT by MedDRA coding, considered related to study product.

7.1.3 Individual Halting Criteria

The study intervention will be discontinued in a participant if any of the following occurs:

1. Laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product if deemed related to the study product by the study investigator. These events must be reported to the medical monitor within 24hrs of investigator awareness.
2. Generalized urticaria (defined as occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product
3. SAE after administration of study product that is considered related to study product
4. Acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product

7.1.4 Criteria for Redosing

Cohorts 1, 2, and 3 will receive a single dose of Sing2016 M2SR H3N2 vaccine or placebo. Cohorts 4, 5, 6, and 7 will receive 2 doses, the first on Day 1 and the second on approximately Day 29. Participants in Cohorts 4, 5, 6, and 7 may receive the second dose if no individual or study halting criteria have been met and the following redosing criteria are met:

Eligibility for Second Dose

Inclusion Criteria

1. Parent/guardian of the participating child continues to agree to permit participation
2. Participant remains in good health as assessed by the principal investigator or other designated study investigator

Exclusion criteria

1. Has a body temperature 38.0°C/100.4°F (oral or axillary) or greater or another acute illness[1] within the 72 hours prior to study vaccination
   [1]A potential participant who is recovering from an acute illness and has residual minimal symptoms, which, in the opinion of the site principal investigator or appropriate sub-investigator, will not likely affect the evaluation of outcome measures is not ineligible.
2. Has any new medical or mental health disease or condition that, in the opinion of the site principal investigator or appropriate sub-investigator, would render study participation unsafe, or would interfere with the evaluation of the responses
3. Has immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy
4. Has a new diagnosis of neoplastic disease
5. Has taken oral, parenteral, or nasal corticosteroids of any dose since the first vaccination
6. Has taken inhaled corticosteroids since the first vaccination
7. Has known HIV, hepatitis B, or hepatitis C infection
8. Has known hypersensitivity or allergy to any components of the study vaccine (sucrose, sodium chloride, phosphate, glutamate), or material in nasal delivery device (polycarbonate, polypropylene, synthetic rubber)
9. Use of aspirin or salicylate containing products since the first vaccination
10. Had documented influenza or received specific influenza antiviral treatment since first vaccination
11. Received any antiviral drug since first vaccination
12. Received a licensed live vaccine since first study vaccination, or plans to receive a licensed live vaccine within the 30 days after the last study vaccination unless provided as part of this study
13. Received a licensed inactivated vaccine since the first study vaccination, or plans to receive a licensed, inactivated vaccine within the 30 days after the last study vaccination
14. Plans to receive an influenza vaccine, other than the one offered as part of participation in this study, within the 30 days following the last study vaccination
15. Received immunoglobulin or other blood products since first study vaccination
16. Received an experimental agent or device since first study vaccination or expects to receive an experimental agent or device during the trial-reporting period

7.1.5 Follow up for Participants who Discontinued Study Intervention

Discontinuation from receipt of Sing2016 M2SR H3N2 vaccine or placebo does not mean discontinuation from the study, and remaining study procedures may be completed as indicated by the study protocol. Participants who receive a single dose of vaccine or placebo, but joined the study in Cohorts 4, 5, 6, or 7, and were thereby assigned to receive 2 doses of vaccine or placebo, will still be offered the seasonal licensed influenza vaccine (IIV4) at the time indicated in the protocol. Such participants will be followed on the schedule of activities in the same manner as participants in Cohorts 1, 2, and 3 (who were assigned to receive only a single dose of vaccine or placebo). If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding after study product administration will be reported as an adverse event (AE).

7.2 Participant Withdrawal from the Study and Replacement

Parents/guardians of participants are free to withdraw their child from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

1. Study non-compliance that in the opinion of the investigator poses an increased risk or compromises the validity of the data
2. If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant, in the opinion of the investigator
3. Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator, might compromise the safety of the participant, interfere with the participant's successful completion of this study, or interfere with the evaluation of responses
4. Participant lost to follow-up

If the parent/guardian of the participant agrees, every attempt will be made to follow all AEs through resolution.

Participants whose parents withdraw them, or who are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) but prior to administration of study product may be replaced, at the discretion of the sponsor.

Given it is crucial to have an adequate pre-vaccination blood specimen from all children who enroll, participants from whom pre-vaccination blood is not successfully collected are not eligible to participate in the study.

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

7.3 Lost to Follow-Up

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

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures (Day -28 to -1, or Day 1):

Screening may occur on Days -28 to -1 or may occur on the day of vaccination, Day 1. During the screening visit, after the study is described to the parent/guardian and child, when appropriate, the parent/guardian will provide written permission and the participant, when appropriate, will provide assent, prior to any study-related procedure. Eligibility will be determined.

Study staff will collect demographic information, medical history, concomitant medications, and either perform or review the physical examination (that is, the physical exam may be performed by the participant's clinical provider, who is not a member of the study team or by a qualified study team member). No safety laboratory tests will be collected. Blood for immune response and nasal washes will be collected at the vaccination visit, prior to vaccination, to establish a baseline. Female participants of childbearing potential will have a urine pregnancy test performed and recorded as negative within the 24 hours prior to vaccination. This test will not be done at a screening visit if the visit is not within 24 hours prior to vaccination. Blood will be collected for immunological assays and a nasal wash will be collected for immunological assays on Day 1, prior to vaccination.

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

If a physiologic parameter, e.g., body temperature, is outside of the protocol-specified range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is unlikely to represent the true body temperature. A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by a malfunctioning or inappropriate measuring device, or for any other reason that the investigator believes has led to a spurious result.

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

No participant may be screened more than twice due to a screening failure result as defined above.

Parents of participants will be provided the results of any abnormal clinical laboratory results (such as a respiratory viral panel result) and will be referred to their primary healthcare provider. Research laboratory results will not routinely be provided to the parent of the participant.

8.2 Efficacy / Immunogenicity Assessments

There are no efficacy evaluations in this study. Immunological evaluations include humoral immune responses, cellular immune responses, and mucosal immune responses following one or two doses of Sing2016 M2SR H3N2 vaccine or placebo. These evaluations also include

immunological evaluations following seasonal influenza vaccine provided to participants after having received 1 or 2 doses of Sing2016 M2SR H3N2 vaccine or placebo.

8.2.1 Humoral Immune Evaluations After Sing2016 M2SR H3N2 Vaccine

Humoral immune evaluations will include HAI, neutralization, anti-HA, and anti-NA antibodies against the homologous vaccine (Sing2016M2SR H3N2) antigens. Responses to heterologous antigens may also be assessed. Analysis of extra-neutralizing function of antibodies to homologous and heterologous strains may also be performed on an exploratory basis according to positive responses obtained for assays described above and specimen availability.

8.2.2 Cellular Immune Evaluations After Sing2016 M2SR H3N2 Vaccine

Cellular immune evaluations will include T-cell responses and antigen-binding B cells to homologous antigen epitopes and may include assessment of responses to heterologous antigen epitopes. Also, these evaluations might include B memory assessment of responses by ELISpot if enough cells are available.

8.2.3 Mucosal Immune Evaluations After Sing2016 M2SR H3N2 Vaccine

Mucosal immune evaluations will include measurement of secretory IgA from nasal wash specimens (sIgA) against homologous vaccine antigens. Additional measurements may include HAI, neutralization, as well as anti-HA and anti-NA IgG and/or IgM antibodies and analysis of extra neutralizing antibody function on an exploratory basis according to specimen availability.

8.2.4 Immune Evaluations After Seasonal Influenza Vaccine (IIV4)

Humoral immune evaluations after seasonal influenza vaccine will include serum HAI and neutralization as well as mucosal secretory IgA to vaccine-containing strains. Additional measurements may include anti-HA, anti-NA antibodies and extra-neutralizing antibody function according to responses obtained in assays described above and specimen availability.
Cellular response analysis after seasonal influenza vaccine will include T-cell responses and antigen-binding B cells and may include responses to heterologous strains; analysis of B memory responses by ELISpot may be included according to cell availability.

8.3 Safety and Other Assessments

Safety will be assessed by evaluations of the frequency and severity of solicited AEs (reactogenicity), unsolicited non-serious AEs, AESIs, NOCMCs, and SAEs.

8.3.1 Solicited AEs (Reactogenicity)

Solicited AEs can be divided into immediate post-vaccination events, local reactogenicity events, and systemic reactogenicity events. The immediate events will be recorded by the study staff in the 20 minutes after each vaccination. All solicited events with corresponding grades as described in Section 8.4.3 will be evaluated. These severity grades include mild, moderate, or severe.

Local and systemic reactogenicity events will then be recorded by the parent/guardian daily from Day 1 to Day 8 after the first vaccine or placebo and the 7 days following the second vaccination for participants in Cohorts 4, 5, 6, and 7 with the use of a memory aid. The local events are those

in and around the nares, where the vaccine is administered. Local and systemic events are described in Table 4, below.

8.3.2 Unsolicited Non-Serious AEs

We will record unsolicited non-serious AEs, both medically attended and not, from the time of vaccination on Day 1 until 28 days after the last vaccination (Day 29 for Cohorts 1, 2, and 3 and approximately Day 57 for Cohorts 4, 5, 6, and 7).

8.3.2.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

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

8.3.3 Adverse Events of Special Interest (AESIs).

Given that there is a theoretical possibility that intranasal influenza vaccines will lead to increased rates of acute onset wheeze and that these events may not always be captured by the other safety assessments, these events will be deemed AESIs. AESIs will be collected from the time of the first study vaccination on Day 1 until individual participant study completion, but with special emphasis paid to the 28 days following each intranasal vaccination. Details on the evaluation of this AESI are found in Appendix B – Evaluation Criteria for Acute Wheezing.

8.3.4 New Onset Chronic Medical Conditions (NOCMCs).

NOCMCs are defined as any new ICD-10 diagnosis that is applied to the participant during the duration of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention. These events will be collected from the time of the first study vaccination on Day 1 until the end of the study period (the final visit done in April of the calendar year following enrollment).

8.3.5 Serious Adverse Events (SAEs).

SAEs will also be collected from the time of the first study vaccination on Day 1 until the end of the study period (the final visit done in April of the calendar year following enrollment).

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

The site PI or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, when they occur in the timeframes described, regardless of relationship to study product. AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether considered intervention-related (21 CFR 312.32 (a)). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational)

product. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the severity or frequency of any pre-existing medical condition increases, it will be recorded as an AE.

Adverse events can be further divided into solicited adverse events and unsolicited adverse events. Solicited adverse events are those for which the study team will specifically query the participant or parent/guardian whether they occurred. Unsolicited adverse events are those events that the participant or parent/guardian reports occurring without being queried about the specific event.

All AEs will be assessed for severity and relationship to study intervention (see section 8.3.3). All AEs, solicited and unsolicited, will be captured on the appropriate data collection form. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness, and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship.

AEs will be followed through resolution.

8.4.2 Solicited Adverse Events

Solicited adverse events are anticipated local and systemic adverse events for which consistent collection of information is desired. For this study, these include local respiratory tract reactogenicity events and systemic reactogenicity events (Section 8.3.1).

8.4.3 Local Reactogenicity

The following Toxicity Grading Scales will be used to grade solicited local (respiratory) and systemic (constitutional) reactions:

Table 4: Grades of Local & Systemic Reactogenicity Assessments, By Age Group

Ages 9-17 Years

Symptoms	Grade 1	Grade 2	Grade 3
Local (Including Respiratory) Reactogenicity			
Rhinorrhea (Runny Nose)	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter
Stuffy nose/congestion	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with breathing through the nose	Unable to breathe through the nose, or prevents daily activity or seeks medical encounter

Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Nasal pain/irritation	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter
Nasal bleeding/ epistaxis	Total duration of all episodes in a 24-hour period ≤30 minutes	Total duration of all episodes in a 24-hour period > 30 minutes, but did not require a visit for a medical encounter	Any bleeding that required visit for medical encounter
Sinus pressure/pain	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Sore throat (may include scratchy or painful throat)	Noticeable but does not interfere with eating and/or drinking	Moderate discomfort. Interferes with eating and/or drinking	Significant discomfort/prevents eating and/or drinking or seeks medical encounter
Cough	Noticeable but does not interfere with daily activity or sleeping	Moderate discomfort/interferes with daily activity or sleeping	Significant discomfort/prevents daily activity or seeks medical encounter
Trouble breathing or shortness of breath	Noticeable but does not interfere with daily activity or not troubled by breathlessness except on vigorous exercise	Moderate discomfort/interferes with daily activity or short of breath with regular movement activities such as when hurrying on a level surface or walking up a slight incline and need to stop	Significant discomfort/prevents daily activity or seeks medical encounter or too breathless to leave the house, or breathless when undressing, preventing normal activities
Systemic Reactogenicity			
Feverishness (may include chills or shivering)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

Fatigue (tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (general unwell feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (general body aches, general muscular pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (general joint pains)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity and/or seeks medical encounter
Flushing	Asymptomatic flushing	Symptoms, some interference with daily activity	Symptomatic, significant interference, prevents daily activity
Decreased activity	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Decreased appetite	Loss of appetite without decrease in oral intake	Loss of appetite associated with decreased oral intake	Loss of appetite without oral intake, seek medical care
Abdominal pain	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	Transient (<24 hours) or intermittent and no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours, or rehydration indicated (e.g., IV fluids)

Vomiting	Transient or intermittent, and no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent vomiting, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Diarrhea	Transient or intermittent, and no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent diarrhea, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Eye pruritus	Transient of intermittent or minimal interference and no intervention	Persistent or frequent episodes, some interference with daily activity	Significant symptoms, prevents daily activity, or seeks medical attention
Eye redness	Asymptomatic eye redness	Symptomatic eye redness, some interference with daily activity	Eye redness prevents daily activity or seeks medical encounter
Allergic Skin Reaction	Pruritus with or without rash, no medical intervention	Localized urticaria, with intervention	Generalized urticaria, anaphylaxis, or angioedema
Fever* - oral or axillary†	38.0°C – 38.4°C (100.4°F – 101.1°F)	38.5°C – 38.9°C (101.2°F – 102.0°F)	>38.9°C (>102.0°F)

- * A fever can be considered not related to the study product if an alternative etiology can be documented.

† Participants must not eat or drink anything hot or cold prior to taking oral temperature

Ages 4-8 Years

Symptoms	Grade 1	Grade 2	Grade 3
Local (Including Respiratory) Reactogenicity			
Rhinorrhea (Runny Nose)	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter

Stuffy nose/congestion	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with breathing through nose	Unable to breathe through nose, or prevents daily activity or seeks medical encounter
Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Nasal pain/irritation	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter
Nasal bleeding/ epistaxis	Total duration of all episodes in a 24-hour period ≤30 minutes	Total duration of all episodes in a 24-hour period > 30 minutes	Any bleeding that required visit for medical encounter
Facial Pain	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Sore throat (may include scratchy or painful throat)	Noticeable but does not interfere with eating and/or drinking	Moderate discomfort. Interferes with eating and/or drinking	Significant discomfort/prevents eating and/or drinking or seeks medical encounter
Cough	Noticeable but does not interfere with daily activity or sleeping	Moderate discomfort/interferes with daily activity or sleeping	Significant discomfort/prevents daily activity or seeks medical encounter
Trouble breathing, shortness of breath	Noticeable but does not interfere with daily activity or not troubled by breathlessness except on vigorous exercise	Moderate discomfort/interferes with daily activity or short of breath with regular movement activities such as when hurrying on the level or walking up a slight incline and need to stop	Significant discomfort/prevents daily activity or seeks medical encounter or too breathless to leave the house, or breathless when undressing, preventing normal activities
Systemic Reactogenicity			

Feverishness (may include chills, shivering, sweating)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue (tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (general unwell feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (general body aches/muscular pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (general joint pains)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity and/or seeks medical encounter
Decreased activity	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Decreased appetite	Loss of appetite without decrease in oral intake	Loss of appetite associated with decreased oral intake	Loss of appetite without oral intake, seek medical care
Abdominal pain	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	Transient (<24 hours) or intermittent, and with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours, or rehydration indicated (e.g., IV fluids)

Vomiting	Transient or intermittent AND no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent vomiting, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Diarrhea	Transient or intermittent AND no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent diarrhea, resulting in orthostatic hypotension or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Eye pruritus	Transient of intermittent or minimal interference and no intervention	Persistent or frequent episodes, some interference with daily activity	Significant symptoms, prevents daily activity, or seeks medical attention
Eye redness	Asymptomatic eye redness	Symptomatic eye redness, some interference with daily activity	Eye redness prevents daily activity or seeks medical encounter
Allergic Skin Reactions	Pruritus with or without rash, no medical intervention	Localized urticaria, with intervention	Generalized urticaria, anaphylaxis, or angioedema
Fever* - oral or axillary†	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

- * A fever can be considered not related to the study product if an alternative etiology can be documented.

† Participants must not eat or drink anything hot or cold prior to taking oral temperature

Ages 6-47 Months

Symptoms	Grade 1	Grade 2	Grade 3
Local (Including Respiratory) Reactogenicity			
Rhinorrhea (Runny Nose)	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter

Stuffy nose/congestion	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with breathing through nose	Unable to breathe through nose, or prevents daily activity or seeks medical encounter
Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort. Interferes with daily activity	Significant discomfort/prevents daily activity
Nasal bleeding/ epistaxis	Total duration of all episodes in a 24-hour period ≤30 minutes	Total duration of all episodes in a 24-hour period > 30 minutes	Any bleeding that required visit for medical encounter
Cough	Noticeable but does not interfere with daily activity or sleeping	Moderate discomfort/interferes with daily activity or sleeping	Significant discomfort/prevents daily activity or seeks medical encounter
Trouble breathing/shortness of breath	Noticeable but does not interfere with daily activity	Moderate discomfort/interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical encounter
Systemic Reactogenicity			
Sleepiness (may include increased napping)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Irritability/Fussiness	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity and/or seeks medical encounter
Decreased activity	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Decreased appetite	Loss of appetite without decrease in oral intake	Loss of appetite associated with decreased oral intake	Loss of appetite without oral intake, seek medical care

Vomiting	Transient or intermittent, and no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent vomiting, resulting in dehydration requiring aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Diarrhea	Transient or intermittent, and no interference with daily activity; minimal interference with oral intake	Frequent episodes with no dehydration and interferes with some daily activity	Persistent diarrhea, resulting in dehydration or aggressive rehydration (e.g., IV fluids) or prevents normal daily activity
Eye pruritus	Transient of intermittent or minimal interference and no intervention	Persistent or frequent episodes, some interference with daily activity	Significant symptoms, prevents daily activity, or seeks medical attention
Eye redness	Asymptomatic eye redness	Symptomatic eye redness, some interference with daily activity	Eye redness prevents daily activity or seeks medical encounter
Allergic Skin Reactions	Pruritus with or without rash, no medical intervention	Localized urticaria, with intervention	Generalized urticaria, anaphylaxis, or angioedema
Fever* - oral or axillary†	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

- * A fever can be considered not related to the study product if an alternative etiology can be documented.

† Participants must not eat or drink anything hot or cold prior to taking oral temperature

8.4.4 Unsolicited Events

All AEs spontaneously reported by the parent/guardian or participant and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded on the appropriate case report form. All reported unsolicited AEs are graded in accordance with the protocol toxicity tables.

8.4.5 Special Reporting of Adverse Events

Following notification from the site PI or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify the

FDA and all investigators (i.e., the participating site PI to whom the sponsor is providing drug under its IND(s) or under any PI's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from the FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

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

8.4.6 Definition of Serious Adverse Events

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

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in inpatient hospitalization, etc.

All SAEs, as with any AE, will be assessed for severity and relationship to study intervention (see section 8.3). All SAEs will be recorded on the appropriate SAE data collection form and eCRF. All SAEs will be followed through resolution by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator). All SAEs will be reviewed and evaluated by DMID and will be sent to the SMC (periodic review unless related) and the IRB/IEC when required.

8.4.7 Classification of an Adverse Event

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

8.4.7.1 Severity of Event

All AEs will be assessed by the study clinician using the following grading system for AE severity.

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

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

AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

8.4.7.2 Relationship to Study Intervention

For each reported adverse reaction, the Principal Investigator or designated clinical investigators will make the determination of relation as defined by:

- Related - The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

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

For this study, the following time periods for reporting of AEs will be followed:

1. Solicited AEs (reactogenicity events): From the time of vaccination on Day 1 until Day 8, for the first vaccination; and from the time of the second vaccination until 7 days later, for those participants who receive 2 vaccinations (Cohorts 4, 5, 6 and 7).
2. Unsolicited non-serious AEs (medically attended and not medically attended): From the time of vaccination on Day 1 until Day 29, for the first vaccination; and from the time of the second vaccination until 28 days later, for those participants who receive 2 vaccinations (Cohorts 4, 5, 6, and 7).
3. AESIs (wheezing episodes): From the time of each vaccination until 28 days later, parents of participating children will be asked at every interaction with study team about wheezing episodes and all episodes will be evaluated as described in Appendix B – Evaluation Criteria for Acute Wheezing. For events occurring beyond 28 days after each vaccination, the parents will have been counseled to notify the study team, and all

relevant data on the wheezing episode will be collected, but there will be no other active attempts at surveillance.

4. NOCMCs, and SAEs: From the time of vaccination on Day 1 until the final study visit (in the month of April of the calendar year following enrollment) for all cohorts.

8.4.9 Adverse Event Reporting

8.4.9.1 Investigators Reporting of AEs

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

8.4.10 Serious Adverse Event Reporting

8.4.10.1 Investigators Reporting of SAEs

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

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

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

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study participant safety and protocol conduct.

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

8.4.10.2 Regulatory Reporting of SAEs

Following notification from the site Principal Investigator or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse event (SUSAR) as an IND safety report to the FDA and will notify all participating site Principal

Investigators (i.e., all Principal Investigators to whom the sponsor is providing drug under its IND(s) or under any Principal Investigator's IND(s)) of potential serious risks from clinical studies or any other source, as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening, the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

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

8.4.11 Reporting Events to Participants

Not applicable

8.4.12 Adverse Events of Special Interest

Early clinical data indicates Sing2016 M2SR H3N2 vaccine is well tolerated in study participants. However, we will collect information about one AESI: wheezing. This AESI will be collected from the time of each study vaccination until the last visit for each individual participant. Details about wheezing and uniform collection of data are found in Appendix B – Evaluation Criteria for Acute Wheezing.

All AESIs will be assessed, recorded, and followed as described above under adverse events. In addition, AESIs will be reported on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

8.4.13 Reporting of Pregnancy

All pregnancies that occur among study participants while enrolled in the study will be reported. If a female becomes pregnant before receiving study product, no product will be given, and her participation will end. Since the only cohort that includes females of childbearing potential is Cohort 1, and participants in this cohort are scheduled to receive a single dose of study product, it is not expected that a female scheduled for 2 doses of study product will become pregnant after one dose. For a female who becomes pregnant, we will request permission to continue to follow her, per protocol, for tolerability, safety, and immunogenicity. She may decline any or all subsequent study follow-up. We will also request to follow her pregnancy and record the outcome. This follow-up will include pregnancy outcome (termination, pre-term birth, term

birth) and newborn outcome (live birth, fetal demise, stillbirth; presence of any congenital anomalies). No in-person visits will be required for pregnancy outcome determination.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

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

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

8.5.2 Unanticipated Problem Reporting

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

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

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

- UPs that are serious adverse events (SAEs) will be reported to the IRB and as per Section 8.4.7 Serious Adverse Event Reporting within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB as per IRB guidelines and to the DCC/study sponsor within three days of the investigator becoming aware of the problem.

8.5.3 Reporting Unanticipated Problems to Participants

Not applicable

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

This study is not designed to achieve pre-determined levels of power or precision to address the primary, secondary, or exploratory objectives. The power and precision allowed by the study's sample size are discussed in Section 9.2, and the general methodology planned to address study objectives is described in Section 9.4. Specific statistical hypotheses and details about planned tables, figures, and data listings will be presented in a separate statistical analysis plan.

9.2 Sample Size Considerations

Table 5 gives the probability of observing one or more safety events, such as a reactogenicity event or an adverse event of a particular classification, for varying sample sizes, given underlying true event probabilities.

Table 5: Probability (%) of observing at least one safety event, given varying underlying event probabilities and sample sizes

Group	**Group Size**	**Underlying Event Probability**			
		0.01% (Very Rare)	**0.1% (Rare)**	**1% (Uncommon)**	**10% (Common)**
Cohorts 5-6 lead-in safety review, Cohorts 6-7 placebo	N=8	0.08	0.797	7.73	57
Cohort 3-4 placebo	N=10	0.1	1	9.6	65.1
Individual placebo group or Cohort 3-4 vaccine group	N=15	0.15	1.5	14	79.4
Individual vaccine group in Cohorts 6-7	N=18	0.18	1.78	16.5	85
Cohorts 1-2 safety review	N=25	0.25	2.5	**22.2**	**92.8**
Individual vaccine group in Cohorts 1-2;	N=30	0.3	3	26	95.8

Cohorts 3-4 aggregate vaccine groups, post first-dose					
Any Sing2016 M2SR H3N2 Vaccine, Cohorts 1-4	N=90	0.9	8.6	59.5	>99.9
Any Sing2016 M2SR H3N2 Vaccine	N=132	1.31	12.4	73.5	>99.9

Among the 25 participants in each of Cohorts 1-2 considered for the safety reviews prior to enrolling the subsequent cohorts, there will be a 22.2% chance of observing at least one uncommon event and a 92.8% chance of observing at least one common event (bolded and underlined in Table 5).

The primary objective of this study is to determine the safety and tolerability of the Sing2016 M2SR H3N2 influenza vaccine, which will be assessed via the incidence of adverse events of different types. While the study was not designed to achieve a pre-determined level of precision, confidence intervals will be estimated for some of the binary endpoints. Table 6 presents the exact 95% Clopper-Pearson confidence intervals that would result from observing varying numbers of events in groups of possible interest.

Table 6: Estimated event probabilities (%) and associated exact 95% Clopper-Pearson confidence intervals resulting from varying numbers of events observed in groups of different sizes.

Group Size	**Number of Events Observed**								
	0	**4**	**5**	**7**	**9**	**12**	**15**	**45**	**66**
Cohorts 5-6 lead-in safety review, Cohorts 6-7 placebo (N=8)	0 (0, 36.9)	**50 (15.7, 84.3)**	62.5 (24.5, 91.5)	87.5 (47.3, 99.7)	-	-	-	-	-
Cohort 3-4 placebo (N=10)	0 (0, 30.8)	40 (12.2, 73.8)	**50 (18.7, 81.3)**	70 (34.8, 93.3)	90 (55.5, 99.7)	-	-	-	-
Individual placebo group or Cohort 3-4 vaccine group (N=15)	0 (0, 21.8)	26.7 (7.8, 55.1)	33.3 (11.8, 61.6)	**46.7 (21.3, 73.4)**	60 (32.3, 83.7)	80 (51.9, 95.7)	100 (78.2, 100)	-	-
Individual vaccine group in Cohorts 6-7 (N=18)	0 (0, 18.5)	22.2 (6.4, 47.6)	27.8 (9.7, 53.5)	38.9 (17.3, 64.3)	**50 (26, 74)**	66.7 (41, 86.7)	83.3 (58.6, 96.4)	-	-
Cohorts 1-2safety review (N=25)	0 (0, 13.7)	16 (4.5, 36.1)	20 (6.8, 40.7)	28 (12.1, 49.4)	36 (18, 57.5)	**48 (27.8, 68.7)**	60 (38.7, 78.9)	-	-
Individual vaccine group in Cohorts 1-2; Cohorts 3-4 aggregate vaccine groups, post first-dose (N=30)	0 (0, 11.6)	13.3 (3.8, 30.7)	16.7 (5.6, 34.7)	23.3 (9.9, 42.3)	30 (14.7, 49.4)	40 (22.7, 59.4)	**50 (31.3, 68.7)**	-	-
Any Sing2016 M2SR H3N2 Vaccine, Cohorts 1-4 (N=90)	0 (0, 4)	4.4 (1.2, 11)	5.6 (1.8, 12.5)	7.8 (3.2, 15.4)	10 (4.7, 18.1)	13.3 (7.1, 22.1)	16.7 (9.6, 26)	**50 (39.3, 60.7)**	73.3 (63, 82.1)

Any Sing2016 M2SR H3N2 Vaccine (N=132)	0 (0, 2.8)	3.0 (0.8, 7.6)	3.8 (1.2, 8.6)	5.3 (2.2, 10.6)	6.8 (3.2, 12.5)	9.1 (4.8, 15.3)	11.4 (6.5, 18)	-	**50.0 (41.2, 58.8)**

For illustration, if zero events of a certain type were observed in an individual vaccine group or in Cohorts 3-4 aggregate vaccine groups after one dose with N=30 participants, true event probabilities above 11.6% could be ruled out at the α=0.025 level, compared to 2.8% in the combined vaccine groups (N=132). Binomial confidence intervals are widest at 50% events observed; the estimates associated with approximately (or exactly) 50% observed events are bolded in Table 6. Within an individual vaccine group or in Cohorts 3-4 aggregate vaccine groups after one dose with N=30 participants, the maximum half-width for a 95% CI would be 18.7%, compared to 10.7% across active vaccine groups in Cohorts 1-4. In the case of substantial loss to follow-up or missed visits, these estimates give an idea of the loss of precision due to decreasing the effective sample size.

While no formal hypothesis testing is planned for this study, some limited statistical testing may be conducted to aid interpretation of the strength of the results. Comparisons would be primarily focused on vaccine to placebo within a given cohort, due to the likelihood of confounding in most results due to age, dose level, or the number of doses (see Section 9.4.1 for details on analysis groupings).

Table 7 presents the probability of a binary event of interest (e.g., adverse event, reactogenicity, seroconversion, etc.) in a vaccine group of size N=30 (Cohorts 1 or 2) that would be detectable with 80% or 90% power given varying event probabilities in a placebo group of size N=15, using two-sided Barnard's exact tests at the α=0.05-level.

Table 7: Detectable probabilities (%) with 80% or 90% power when comparing binary event probabilities between groups with two-sided Barnard's exact tests with α=0.05.

Probability in Placebo Group (N=15)	**Detectable Probability in Vaccine Group (N=30)**	
	80% power	**90% power**
0.1% (Rare)	29.0	32.9
1% (Uncommon)	31.5	36.5
10% (Common)	48.4	54.4
50% (Very Common)	88.2	92.4

There would be 80% power to detect a difference of 29% between vaccine and placebo groups for a rare event (0.1% probability) in the placebo group, compared to a difference of at least 48% for a common or very common event in the placebo group.

9.3 Populations for Analyses

- The enrolled population will include all participants enrolled and randomized

- The Safety population will include all participants who receive at least one study influenza vaccination.
- The Modified Intent-to-Treat (mITT) population will consist of all participants who received study influenza vaccination and reported baseline and at least one post-baseline immunogenicity result.
- The Per-Protocol (PP) population will include participants in the mITT population with the following exclusions of data:
    - Data from participants determined to be ineligible at baseline (determined thereafter)
    - Data from participants in Cohorts 4, 5, 6, and 7 subsequent to missed second study vaccinations
    - Data from any visit that occurs substantially out of window as defined in the SAP
    - Data from all visits subsequent to major protocol deviations, as defined in the SAP, that could impact the validity of later data, such as:
        - Receipt of immunosuppression or any medications that may be associated with impaired responsiveness
        - Receipt of any non-study investigational drug/investigational vaccine/licensed vaccine

The enrolled population will be used for summaries of participant disposition as well as participant demographics and baseline characteristics. The Safety population will be used for all analyses of adverse events and reactogenicity. Immunogenicity analyses will be done in the mITT population, and if sufficient data (>10% of secondary analysis observations) would be excluded by the PP exclusions, the PP population will be used for sensitivity analyses. Certain analyses may be done on subsets of these populations, such as by-dose analysis, where applicable, and these would be specified in the analysis plan.

9.4 Statistical Analyses

The primary clinical database for this study will consist of safety (adverse events and reactogenicity) and baseline/demographic data. Once the last participant has completed the final visit, the primary clinical database will be cleaned, monitored, and locked. After clinical database lock and receipt of secondary immunogenicity data, a set of topline tables will be generated by the Statistical and Data Management Coordinating Center (SDMCC), including summaries of clinical safety and secondary immunogenicity data. The topline report will be made available to the study team for planning subsequent trials and may be presented in a public forum or used for publication in collaboration with lead PI. These analyses will be considered final and will be included in the Clinical Study Report (CSR) as well. The CSR, comprised of the final analyses of safety and available immunological data, will be subsequently completed. Any available data from the exploratory endpoints may also be included. Additional exploratory endpoint data not available at the time of CSR preparation may be included in one or more addenda to the CSR, manuscript(s), or other report. A formal Statistical Analysis Plan (SAP) that specifies all planned analyses will be finalized prior to generating the topline report and CSR.

In addition to the descriptive analyses of study data for SRC and SMC review, an interim analysis is planned for while the study is ongoing to allow for an early assessment of the humoral immunogenicity of the study vaccine and to aid in decision-making regarding future studies. These analyses would not affect the conduct of this trial via early stopping, and participant-level unblinding will not occur until the clinical database is locked. Additional details on interim analyses are given in Section 9.4.6.

The formal SAP will elaborate on the analyses described here.

9.4.1 General Approach

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

Generally, summaries will be presented by analysis group including cohort and vaccination group (i.e., Cohort 1 – active, Cohort 1 – placebo, Cohort 2 – active, etc., called "group" throughout this section). Post-first dose safety and immunogenicity results for active Cohorts 3+4 will be combined as both cohorts receive the same dose and belong to the same age group. Additional analysis groupings may be specified in the analysis plan.

For safety endpoints, summaries may also include a column for all participants receiving active study vaccination and all participants receiving placebo. Data listings will be presented sorted by clinical site or other grouping variable, participant, and then by visit number within participant, where applicable. All tables will be annotated with the total population size relevant to that table, including any missing observations. The analysis population for each exhibit will be clearly indicated.

Prior receipt of IIV4, season of prior receipt of IIV4, and time between prior receipt of IIV4 and study vaccination, as well as receipt of the COVID-19 vaccine during the study period and the timing of receipt of the COVID-19 in relation to study vaccination may be considered in exploratory analyses to adjust for potential confounding.

The study was not designed for any formal group comparisons, but hypothesis testing may be performed for some endpoints and would be considered exploratory. Adjustments for multiplicity or more stringent thresholds for significance than the typical α=0.05 type-I error may be used for immunogenicity analyses involving numerous endpoints. Details on any hypothesis testing will be described in the standalone SAP.

9.4.2 Analysis of the Primary Endpoint(s)

The primary objective of the study is to assess the safety and tolerability of 1 or 2 doses of the study vaccination, as determined by the incidence of solicited reactogenicity events, unsolicited

serious and non-serious adverse events, adverse events of special interest, and new-onset chronic medical conditions.

Safety objectives will be assessed in the Safety population. The vaccine and placebo groups from Cohorts 3 and 4 will be combined for post-first dose analyses and presented separately after the second dose in Cohort 4. Solicited local and systemic reactogenicity events will be summarized via the number and percentage of participants reporting each event, and any solicited reactogenicity event, within the 7 days following each dose in each group. These will be tabulated by severity, by dose, and post-any dose. Exact 95% Clopper-Pearson confidence intervals for the probability of each event post-each and any dose will be computed for each group. The frequency of each solicited event will be summarized as well. Severity will be determined as described in Section 8.4.3.

Non-serious unsolicited adverse events and adverse events of special interest occurring within the 28 days post-each dose and post-either dose will be summarized by MedDRA system organ class (SOC) and preferred term (PT), severity, and dose, as well as post-any dose. Serious adverse events and new-onset chronic medical conditions reported through the end of the study period will be summarized similarly. Abnormal clinical laboratory values will be considered unsolicited adverse events and graded as in Section 8.4.2, and these will be summarized separately. For unsolicited events, summaries will also be made by relationship to study vaccine, as described in Section 8.4.4.

Adverse events and clinical laboratory results will be presented in data listings as well.

9.4.3 Baseline Descriptive Statistics

Baseline and demographic characteristics will be summarized by group, and separately by clinical site, with summary statistics as described in Section 9.4.1. These characteristics will include age, sex, ethnicity, race, and prior receipt of seasonal vaccination.

9.4.4 Analysis of the Secondary Endpoint(s)

Humoral immunogenicity of the study vaccination will be assessed via hemagglutination inhibition (HAI) in serum, neutralization (Neut) in serum; and secretory IgA (sIgA) as measured by the binding antibody multiplex assay (BAMA), assays against the homologous strain of H3N2 M2SR-like virus. These assays will be analyzed separately by analysis groups defined in Section 9.4.4.

HAI and Neut titers will be summarized by analysis group at baseline and Day 29or Day 57 using the geometric mean (GMT) and the number and proportion of participants reporting a titer ≥ 1:40 (putative seroprotection for HAI). For post-baseline time points, the geometric mean fold-rise (GMFR) and the number and proportion of participants reporting at least 2-fold and at least 4-fold rises from baseline will also be presented. Associated 95% confidence intervals will be computed for each of these, using the t-distribution for GMTs and GMFRs and exact Clopper-Pearson binomial CIs for the binary endpoints. Bootstrap confidence intervals may be calculated for continuous endpoints instead if the t-distribution is determined to be

unsuitable for continuous endpoints. Additional details of analyses of secondary endpoints will be provided in the SAP.

9.4.5 Analysis of the Exploratory Immunogenicity Endpoints

The exploratory objectives of the study involve further characterization of the immunogenicity of the study vaccine and the seasonal IIV4 vaccine given to each participant after the study vaccination(s). These will include additional assessments of humoral responses as well as cellular immunogenicity (T cell responses).

For analysis of exploratory immunological endpoints, immune responses will be described by time point and analysis group, similarly to the summaries of secondary immunological outcomes. For titer data, geometric means will be computed for each group at each time point (GMTs), and geometric mean fold-rise (GMFR) at post-baseline visits will be calculated as well, where applicable. Confidence intervals corresponding to these statistics will be computed for each group, but formal hypothesis testing is not planned for descriptive summaries of exploratory endpoints. Other immunological endpoints will include frequencies of T cell subsets, which will be described by mean or median frequencies within each group and the associated 95% CIs depending on distributional properties. Additional details will be provided in the SAP.

Responses in serum will be compared with analogous responses in plasma. These analyses will be described in the SAP.

Exploratory analyses of humoral immunogenicity assays will include statistical modeling of seroprotection and/or appropriately transformed titers over time, while accounting for age, dose level, sex, and prior seasonal vaccination status. These analyses will be described in the SAP, along with further details for analyses of exploratory immunogenicity endpoints and exploratory analyses of secondary immunogenicity endpoints.

9.4.6 Planned Interim Analyses

9.4.6.1 Interim Safety Analyses

There are two safety committees that will be convened for this study: The Safety Review Committee (SRC) and the Safety Monitoring Committee (SMC).

The SRC will review blinded safety data to allow for progression through the cohorts as described in the study design section, Section 4.1. The SMC will review safety data if criteria for progression to enrollment of a subsequent cohort is not met, a halting rule (as defined in Section 1.1) occurs, or as regularly scheduled per the separate SMC charter.

Interim safety reviews may include enrollment and demographic information, clinical laboratory tests, and AE/SAEs. During SMC reviews, additional data may be requested by the SMC. The reviews will be blinded to study assignment, with unblinded study assignments available upon SMC request. As an outcome of each SMC review, the SMC will make a recommendation to continue, modify, or terminate this study.

9.4.6.2 Interim Analyses

As described in Section 9.4, an interim analysis focused primarily on immunogenicity will be conducted while the study is ongoing. These analyses, including primary, secondary and exploratory endpoints, may be made available to the pharmaceutical partner for planning for future studies, with no public dissemination of results, and will not be used to make any decisions concerning the conduct of this trial. Analyses from specimens (serum, plasma, nasal washes) collected through the Day 29 visit (Visit 4) for Cohorts 1 to 3 and through D57 visit (Visit 4C) for others will be presented in aggregate by cohort and vaccine/placebo. Interim safety analyses through the Day 29 visit (Visit 4) for Cohorts 1 to 3 and through D57 visit (Visit 4C) for others will only be presented aggregated by cohort, to avoid the possibility of unblinding due to rare adverse events occurring only in active vaccine or placebo groups.

9.4.7 Sub-Group Analyses

Most analyses will be conducted by age group (i.e., by Cohort). Other exploratory analyses may be conducted by sex or by previous seasonal vaccination status, to determine potential confounders other than age that could impact safety, reactogenicity, or immunogenicity of the study vaccination.

9.4.8 Tabulation of Individual Participant Data

Safety data, reactogenicity data, and immunogenicity data will be presented in listings, as described in Section 9.4.1.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

Each site principal investigator will obtain IRB approval for this protocol and any amendments to be conducted at his/her research site(s) followed by DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, informed consent, and associated documents prior to the recruitment, screening, and enrollment of participants. Study activities may not begin until all approvals including both DMID as well as IRB are attained.

10.1.1 Informed Consent Process

Before any study procedures are performed, parental permission and participant assent, where appropriate, will be obtained and documented for all study participants. All participants undergo a consent process that includes both a verbal discussion of study information as well as written documentation. The key information provided will include the purpose of the study, the procedures and experimental aspects of the study, study interventions/products, probability for random assignment to treatment groups, risks and discomforts, the expected duration of the participant's participation in the trial, any expected benefits to the participant, and alternative treatments and procedures that may be available to the participant. The explanation will be organized and presented in lay terminology and using language that facilitates understanding

why one might or might not want to allow one's child to participate or, in the case of assenting minors, why they themselves might or might not want to participate.

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

The parents/guardians of participants will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations, and that, by signing a written parental permission form, the parent/guardian of the participant is authorizing such access.

The parents/guardians of participants, and participants themselves when assenting, will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends, or legally authorized representative, or think about it prior to agreeing to participate.

Parental permission forms and assent forms for children will be IRB-approved, and participants will be asked to read and review the written forms. Parents/guardians of participants will sign the parental permission form, and participants will sign the written assent form, as required, prior to starting any study procedures being done specifically for this trial. Once signed, a copy of the forms will be given to the parent/guardian of the participant(s) for their records.

New information will be communicated by the site principal investigator to parents/guardians of participants who provide permission for their children to participate in this trial in accordance with IRB requirements. The parental permission document and assent forms will be updated, and parents/guardians of participants will be asked to provide updated permission per IRB requirements, if necessary. Participants may be asked to re-assent, if required by the IRB, following updated information.

The participant's parent or guardian will be asked to consent for future use of specimens for secondary research; genetic testing on these samples may be performed. Please refer to Section 10.1.4.1.

10.1.1.1 Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)

The study proposes to enroll participants 6 months to 17 years of age. Investigators or their designee will conduct the consent process with the parent(s)/legal guardian, who will be given an IRB/IEC-approved permission form, which may be referred to as a consent form, to read, review, and sign prior to any study procedures. In addition, assent will be obtained in accordance with the approval by the site or central IRB.

The participant who reaches the age of majority or becomes emancipated during the study will be consented at the next visit prior to study procedures being performed. When no further visits are planned but the participant's participation is ongoing, the consent will be obtained via IRB/IEC-approved processes.

10.1.1.2 Other Informed Consent Procedures

The rights and privacy of human participants who participate in genomic or phenotypic research studies will be protected at all times. The consent process, including relevant language in the ICF will provide an explanation of the potential risks to the parents of the individual study participants. The consent will include whether individual participant data will be shared through a NIAID-designated controlled access data repository. Clinical metadata, genomic, or other datasets or a subset of the clinical and other metadata that may potentially identify human participants will not be released in unrestricted databases. Parents of participants will be informed that the evolution of genomic technology and analytical methods raises the risk of re-identification, even when specimens are de-identified. The parents of the participants will be asked to consent specifically to genetic testing that may be performed under secondary human participant research.

10.1.2 Study Termination and Closure

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

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Regulatory authorities determine it will be terminated

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

The sponsor will notify regulatory authorities as applicable.

10.1.3 Confidentiality and Privacy

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

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

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

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released to meet the requirements of the Federal Food and Drug Administration (FDA).

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

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

Participant study information will not be released without the written permission of their parent/guardian, except as necessary for oversight by the protocol Principal Investigator or designee, the study funders, and applicable regulatory bodies.

All research laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality.

10.1.4 Future Use/Secondary Use of Stored Specimens and Data

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

10.1.4.1 Samples for Secondary Research

Leftover research samples not required for the laboratory testing specified in this protocol will be stored for potential future studies with the subject's consent. Specimens will be coded. Any future testing laboratory will not have access to the code, and therefore will not be able to identify study participants. Genetic testing on these samples may be performed with corresponding permissions as part of the Biorepository consent (DMID 19-0025).

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

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

In addition to this protocol, participants and their parent/guardian will be asked to consent to a separate biorepository protocol (DMID 19-0025) for the use of residual samples and associated data in future research.

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

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

Reports from secondary research will not be kept in the participants' health records or shared with participants or their parents/guardians, unless required by law. Reports will not be sent to the specimen repository. The participant's or the parent/guardian's decision for secondary research can be changed at any time by notifying the study doctors or nurses in writing. If the

participant changes his/her decision, the samples will be destroyed if the samples have not been used for research or released for a specific research project.

10.1.4.2 Data Sharing for Secondary Research

Data from this study may be used for secondary research. All of the individual participant data collected during the trial will be identified by a coded number that is linked to a participant through a code key maintained at the clinical site. The code key is kept confidential and will not be transmitted off site. All of the individual participant data collected during the trial will be made available after deidentification. The Protocol, Statistical Analysis Plan, and Analytic Code will also be made available. These data will be available immediately following publication, with no end date.

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

10.1.5 Key Roles and Study Governance

This study is sponsored by DMID. The Principal Investigator, DMID Clinical Project Manager, DMID Medical Officer, and DMID Scientific Lead are listed on the cover page. Other study team members and roles are listed in the Manual of Procedures (MOP).

10.1.6 Safety Oversight

The SRC Safety Review Committee consists of the PI at each participating site (or their delegate), representatives of the sponsor (at least the Medical Monitor and the Medical Officer), representatives of the manufacturer (at least the Medical Lead or delegate), and representatives of the SDMCC. The SRC will review the data required to allow progression from cohort to cohort. These reviews occur as described in the study design section, Section 4.1. Ad hoc reviews may also be called for by the investigators or sponsor. Decisions are dependent on the failure to meet halting criteria when the required follow-up data are available. The SRC deliberations may occur by teleconference, videoconference, or email. If the SRC determines that the study data have met the criteria necessary to begin a subsequent cohort, the sponsor will notify the sites of the determination. If the SRC is unable to determine that the criteria necessary to begin a subsequent cohort have been met, or if halting rules have been met, the SMC will convene.

The SMC is an independent group of at least 3 experts that monitors participant safety and advises DMID. SMC members will be separate and independent of study personnel participating in this trial and should not have scientific, financial, or other conflicts of interest related to this trial. The SMC will consist of members with appropriate expertise to contribute to the interpretation of data from this trial. A quorum will consist of a simple majority.

The SMC will hold an organizational meeting prior to enrollment. At this meeting, the SMC will review the charter, protocol, ICF, and safety report template.

The SMC will conduct the following reviews:

1. Review of safety if a halting rule is met

2. Review of safety if the SRC requests a review
3. Review of safety, tolerability, and trial progress at the following times: after the first season of vaccinations, after the second season of vaccinations, at the conclusion of the trial
4. Ad hoc review as needed when requested by the sponsor, investigators, or SMC

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review applicable data including, but not limited to, enrollment, demographics, dosing, data, laboratory data, and safety data at scheduled time points during this trial as defined in the SMC charter. The SMC will review blinded aggregate data in the open session of the SMC meetings. Unblinded data (grouped by treatment) may be reviewed in the closed session only. When possible, these data will be "aliased" in that the SMC will review the data by group, but the identity of the group will not be divulged. The SMC may, if necessary, review grouped data in which the identities of the groups are divulged.

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

10.1.7 Clinical Monitoring

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

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

10.1.8 Quality Assurance and Quality Control

To ensure the reliability of study data, the site will develop a Clinical Quality Management Plan (CQMP). The CQMP will describe routine internal quality control (QC) and quality assurance (QA) activities for the purposes of measuring, documenting, and reporting study conduct, protocol adherence, human participant protections, and reliability of the protocol-driven data collected. A process for addressing in a timely manner any data quality issues (for example,

collecting, recording, and reporting data), systemic issues (for example, protocol conduct, non-compliance, human participant protections), and implementation of Corrective and Preventative Action (CAPA) procedures.

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

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Clinical research data (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments), and clinical laboratory data will be abstracted from the source documentation and/or collected on source document worksheet by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. Alternatively, direct data entry may be employed. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

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

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data. The IND sponsor is responsible for review of data collection tools and processes, and review of data and reports. Adverse events will be coded according to the MedDRA dictionary version 23.0 or higher. At the end of the study, a copy of all datasets including annotated case report forms and data dictionary will be provided to DMID.

A separate SDSP will be developed which describes the technical recommendations for the submission of human study data and related information in a standardized electronic format throughout product development.

10.1.9.2 Study Record Retention

Study-related records, including the regulatory file, study product accountability records, consent forms, participant source documents and electronic records should be maintained for a period of 2 years following the date a marketing application is approved for the investigational product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. These documents should be retained for a longer period, however, if required by local policies or regulations. No records will be destroyed without the written consent of DMID. Consent forms with specimen retention linked to identifiable specimens will be maintained for as long as the specimens remain in identifiable format, and a minimum of three years after use of the identifiable specimens in nonexempt human participant research.

10.1.9.3 Source Records

Source data are all information, original records of clinical findings, observations, or other activities documented in a clinical trial necessary for the reconstruction and evaluation of the trial. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP, regulatory, and institutional requirements. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

For this trial, investigators may, when necessary, use the electronic medical record as source data, but most of the information required will be collected from the parents/guardians and as part of the conduct of the study.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, any process that is noted in the protocol and refers to details in the MOP, or GCP requirements. Noncompliance may be either on the part of the participant, the investigator, or the study site staff. Following a deviation(s), corrective actions should be developed by the site and implemented promptly. All individual protocol deviations will be addressed in participant study records.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the protocol deviation reporting procedures. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the participant's chart if the deviation is participant specific.

10.1.11 Publication and Data Sharing Policy

10.1.12 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations: National Institutes of Health (NIH) Public Access Policy. This policy which ensures that the public has access to the published results of NIH-funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.13 Genomic Data Sharing Plan

Not applicable

10.1.14 Publication

Following completion of the study, the Lead Principal Investigator is expected to publish the results of this research in a scientific journal. Data from the clinical trial may also be presented in CIVICs program seminars and the CIVICs annual meeting, subject to approval by DMID, or

used for publication as determined by the PI in collaboration with the study team and in accordance with DMID data sharing policy and the Clinical Trial Agreement. This study will adhere to the publication and data sharing policies and regulations of the sponsor: NIH Public Access Policy. This policy ensures that the public has access to the published results of NIH-funded research. As such, the final peer-reviewed journal manuscripts will be accessible to the public on PubMed Central no later than 12 months after publication.

10.1.15 Conflict of Interest Policy

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

10.2 Additional Considerations

10.2.1 Research Related Injuries

If it is determined by the participating site PI that an injury occurred to a participant as a direct result of the tests or treatments that are performed for this trial, then referrals to appropriate health care facilities will be provided to the participant and his or her parent/guardian. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site, such as giving emergency medications to stop immediate allergic reactions due to the study vaccine. No financial compensation will be provided to the participant by NIAID, NIH, or the participating site for any injury suffered due to participation in this trial.

10.3 Abbreviations

AE	Adverse Event
CAPA	Corrective and Preventative Action Plan
CFR	Code of Federal Regulations
CI	Confidence Interval
CMP	Clinical Monitoring Plan
CMS	Clinical Material Services
CRF	Case Report Form
CROMS	Clinical Research Operations & Management Support
CSR	Clinical Study Report
DCC	Data Coordinating Center
DMID	Division of Microbiology and Infectious Diseases
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HA	Hemagglutinin

HAI	Hemagglutination inhibition
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
MITT	Modified Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to participants)
NA	Neuraminidase
NAI	Neuraminidase Inhibition
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RVP	Respiratory Virus Panel
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SRC	Safety Review Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
2.0	02/11/2022	Minor changes incorporated including: Clarification on date when the study will start, acceptable mechanisms of contraception and co-enrollment of Cohort 4 & 5. The protocol was also further updated for consistency and additional clarifications provided.	The protocol builds in additional conditions that may impact study conduct for further clarity/guidance for clinical sites
3.0	09/07/2022	Minor changes incorporated including: Additional clarification to avoid enrolling individual participants with a history of	The protocol builds in additional clarification to study procedures as well as a decreased sample size given

		wheezing episodes and including a narrower window for study visit 2 related to evaluation of wheezing reported after receipt of study vaccine. Decrease in sample size for Cohort 4 from 45 to 25 with corresponding changes in study schema and Statistical Considerations section. Incorporated study halting criteria if influenza activity is greater than 10% locally or regional ILI activity is high. Other minor edits incorporated including correction of window for receipt of seasonal influenza vaccine (in Schedule of Activities), wording related to dose preparation of IP and evaluation of wheezing.	it does not impact probability of identifying unexpected adverse reactions to study vaccine.
4.0	05/26/2023	Change the timing of and required reviews by the SRC after Cohort 5. Descriptions of the antibody test for neuraminidase antibodies has been changed from NAI, a specific type of assay, to anti-NA, to allow for anti-neuraminidase assays other than NAI	Allow administration of first dose in Cohort 6 after safety data of first dose in Cohort 5 and allow for second doses in Cohort 6 to begin after at least 6 of 8 in Cohort 5 have completed through the third day after their second dose. To accommodate the optimal assay/s to measure antibodies against neuraminidase
5.0	12/7/2023	Minor edits in the study design section indicating Cohorts 5-7 will not be enrolled, clarification of endpoints and removal of ISM language	Changes are made to accommodate FDA and other regulatory bodies requirements given non-enrollment in Cohort 5-7 due to a number of non-safety related contingencies (timelines, budgets, recruiting, and lack of additional vaccine lots).

			Other changes include more clarity on proposed secondary study objectives and associated secondary and exploratory endpoints. Other minor changes include clarification on anticipated interim analysis and publication plans. Additionally, given the sponsor no longer uses an ISM, ISM language has been removed from the protocol.

11. APPENDIX

11.1 Appendix A – Blood and Fluid Collection Volumes by age group

Table 8: Blood and Fluid Collection volumes by Age

Visit number	1	4 OR 4C	6	**Target Volume by Day for Serum OR Whole Blood**	**Max Volume by Day for Serum OR Whole Blood**
Target day	1	29 OR V4+28d	V5+28d		
First day of window	1	26 OR V4+25d	V5+25d		
Final day of window	1	32 OR V4+31d	V5+31d		
Visit Type	Vaccine	Vaccine OR Follow	Follow		
Visit venue	Clinic	Clinic	Clinic		
Procedures	*per visit*				
Serum Vol. (*required for all ages)*	5ml			**Serum**	**Serum**
Additional Serum Vol. 6mo – 4yo 5yo - 8yo 9yo – 17yo	 None 5 ml (if possible) 5 ml			 6mo-4yo: 5ml 5yo-8yo: 5ml 9yo-17yo: 10ml	 6mo-4yo: 5ml 5yo-8yo: 10ml 9yo-17yo:10ml
Whole Blood Vol. 6mo – 4yo 5yo – 8yo 9yo – 17yo	 8.5ml (if possible) 8.5 ml 8.5 ml			**Whole Blood** 6mo-4yo: 0ml 5-8yo: 8.5ml 9-17yo: 17ml	**Whole Blood** 6mo-4yo: 8.5ml 5-8yo: 8.5ml 9-17yo: 17ml
Additional Whole Blood Vol. 6mo – 4yo 5yo – 8yo 9yo – 17yo	 None None 8.5 ml				
Target Volume by Day 6mo – 4yo 5yo – 8yo 9yo – 17yo	 5.0 ml 13.5 ml 27.0 ml			**Total Target by Age for study** 6mo – 4yo -15ml 5yo – 8yo – 40.5ml 9yo – 17yo – 81ml	
Max Volume by Day 6mo – 4yo 5yo – 8yo 9yo – 17yo	 13.5 ml 18.5 ml 27.0 ml			**Total Max by Age for study** 6mo – 4yo – 40.5ml 5yo – 8yo – 55.5ml 9yo – 17yo – 81ml	
Non-Blood Sample					
Nasal Lavage	5-10 ml			15-30 ml across all ages for study	

11.2 Appendix B – Evaluation Criteria for Acute Wheezing

The Brighton Collaboration has set forth their recommendations for the evaluation of wheezing following vaccination[20]. This study will, in general, conform with those recommendations.

Definition.

Acute wheeze is the sudden onset of adventitious breath sounds that are high pitched or whistling. They are generated by turbulence of air in the intrathoracic airways due to airflow limitation. Acute wheeze has the following characteristics:

1. The sounds must be of sudden onset (unexpectedly and without warning, leading to a marked change in a participant's previously stable condition).
2. The sounds must be "adventitious" which means extra or additional sounds heard over the participant's normal breath sounds.
3. The sounds must involve high-pitched breath sounds, which may sometimes be "whistling" in nature.
4. The sounds must be audible on auscultation with a stethoscope by a healthcare provider and the sounds must be audible during expiration. (There may also be inspiratory sounds, but there must not be *only* inspiratory sounds.)

Although parents and caregivers of children, or children themselves, may provide the study team or their healthcare providers with complaints they term "wheezing" or other complaints that lead to a healthcare provider diagnosing "acute wheeze", the symptom of "wheezing" or any other symptom that a parent or caregiver of a child, or a child him- or herself, reports or endorses, will not be deemed as adequate to meet the case definition (although it is often the impetus for evaluation by a healthcare provider or the research team).

When all the above criteria have been met, the child will be termed to have definite acute wheeze. When the study team is provided with information after the time when confirmation of acute wheeze is possible, such as at a future research follow-up visit, and the documentation of the episode cannot confirm or deny that it met the criteria, then the investigator, using his/her judgment, may record the event as "possible acute wheeze". For example, a parent states that the child has a respiratory event that led to a health care visit and at the visit the child was given a bronchodilator, but the documentation required to meet the criteria were not recorded or are not available, such an even would be deemed "possible acute wheeze."

Timing.
Enhanced surveillance of episodes of acute wheeze will occur in the 28 days after intranasal administration of study product. During the 28-day period post-vaccination, families will be given instructions to notify the research team immediately for all illnesses, including "wheezing", respiratory symptoms such as cough, sneezing, rhinorrhea, tachypnea, chest indrawing, etc. at each encounter with the study team and via a weekly eDiary check conducted within +/- 3-day window. If symptoms trigger an evaluation, a research clinician will conduct the evaluation, if possible. If an external physician completes the evaluation, data will be retrieved from the medical records of the participant. The evaluation will include a standard set of clinical variables and a respiratory viral panel. Following this period of enhanced surveillance for acute wheeze, the study team will continue to collect information on all reports of wheezing until the end of each child's participation, which spans until the last clinical evaluation of the participant in the following year (April of the following year). During this period, families will be instructed to let the study team know about any wheezing episodes but not all respiratory events, such as rhinorrhea and sneezing. Information on the wheezing events, including respiratory virus panel (RVP) results, if conducted as part of routine care, will be collected via chart abstraction.

Data collection.

For each of these episodes, the participant will have collected, preferably by the research team, but if not possible then by an outside provider, a standard set of variables. If not all the variables are collected by an outside provider, the study team will record the available data. When possible, the research team will evaluate the acute episode. If not all the variables are available, to determine if the event met the criteria for definite acute wheeze, the investigator may report it as possible acute wheeze. If a complete RVP is not available but another microbiologic test was performed and provides an alternate explanation then this will be considered acceptable in lieu of investigator initiated RVP.

Determining whether the episode meets or does not meet the definition.
Episode evaluations will first determine if the definition of definite acute wheeze has been met, as defined above. Variables necessary to make that determination are the following: 1. Acute onset episode (y/n), 2. Auscultation by a healthcare provider using a stethoscope reveals breath sounds that are high-pitched/whistling, adventitious (y/n), and 3. The sounds are heard during expiration (y/n).

Standard set of variables to be collected.
If the definition of acute wheeze *is not* met, routine collection of AE variables, when in the time frame stated in the protocol for AEs, will be done.

If the definition of acute wheeze *is* met, the following information will be collected, in order to characterize the acute wheeze episode:

1. Date of onset of symptoms that led to evaluation.
2. Date of meeting the case definition of acute wheeze
3. Date when the AE resolves – this will be determined when symptoms resolve and does not require repeat physical exams.
4. Relationship of person reporting the symptoms that prompted evaluation
5. Role (investigator, outside healthcare provider, etc.) of person performing auscultation and respiratory evaluation.
6. Accompanying respiratory findings:
    a. Respiratory Rate in breaths per minute (graded into normal or tachpneic, by age[1])
        [1]The upper limit by age is as follows: 6–11mo - 38 breaths/min; 1–3 yo - 30 breaths/min; 4–9yo - 24 breaths/min; 10–13yo - 22 breaths/min; 14–17yo – 20 breaths/min [21]
    b. Inspiratory: Expiratory ratio (less than or equal to 1:3 [normal] or 1:3 or greater [prolonged])
    c. Lower chest wall indrawing (yes or no)
    d. Central cyanosis (yes or no)
    e. Oxygen saturation by pulse oximetry (actual value and graded as <92% [significant hypoxia] or 92% or more [normal or mildly abnormal])
    f. Inability or significant difficulty in breastfeeding, drinking, talking, based on age- (inability, difficulty, neither)
    g. Altered mental status/confusion as determined by the healthcare professional (yes or no)
7. Accompanying non-respiratory findings:
    a. Temperature (including type of thermometer)

   b. Blood pressure (if available)
8. Treatment:
   a. Type
   b. Dose
   c. Duration
9. Outcome
10. Is this an SAE?
11. Exposures other than the investigational product that could potentially be the etiology: infection, environmental, food
12. Results of RVP, including COVID-19 test, or other infectious disease diagnostics performed.

Severity Grades

All episodes of acute wheeze will be graded based on the most severe time point within the single episode. Clinical discretion may be employed to determine when an event is deemed a new event, since signs and symptoms may come and go during a single wheezing event. The grading of the event should be as follows.

Mild = Grade 1 = "Wheeze without respiratory distress or danger signs". Definition: wheeze only- that is, without any of the additional findings in Grades 2 or 3.

Moderate = Grade 2 = "Wheeze with respiratory distress but without danger signs". Definition: wheeze plus one or both of the following: lower chest indrawing, I:E > 1:3, but none of the additional findings in Grade 3.

Severe = Grade 3 = "Wheeze with danger signs". Definition: wheeze plus one or more of the following: oxygen saturation < 92%, central cyanosis, altered mental status/confusion, inability to breastfeed, drink, or speak (depending on age)

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