Statistical Analysis Plan

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

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

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# 1. STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

The objective of this analysis plan is to describe the planned study analyses. Where the protocol and this SAP disagree, the SAP takes precedence. This SAP is to be finalized prior to locking the clinical database.

## 1.1. Statistical and Analytical Plans

### 1.1.1. Definitions and General Considerations for Data Analysis

1. This Statistical Analysis Plan (SAP) is based on Clinical Trial Protocol FluGen-H3N2-V002-V005 dated 10-May-2021 (v2.0).
2. All programming and calculations will be performed using SAS statistical software, version 9.4 or later.
3. Study Day 1 is the day of randomization and 1st IP administration; QIV administration is planned 28 Days after IP administration
4. Baseline (IP Baseline) is the last observation prior to IP administration, at the datapoint, not the record level. For example, for laboratory values, individual repeat tests values will be used for baseline when the entire panel may not have been repeated.
5. Summaries will be presented by treatment and age group and for all ages combined. Cohorts 2 and 3 will not be presented separately.
6. This study is not powered to detect differences in safety or immunogenicity between active and placebo groups. The sample size of 120 (80 active subjects and 40 placebo subjects) was chosen empirically based on studies of other vaccines in early-stage development.
7. There is no plan for test of hypotheses; p-values generated for immunogenicity are descriptive only and not adjusted for multiplicity.
8. For transparency and traceability, raw data, not results from calculations or corrections are to be transferred from external vendors. Differences between data transferred versus data as analyzed, will be documented in the CSR.
9. The clinical database will be locked prior to final analyses of immunogenicity assays. Details regarding these analyses may be included as amendments to this SAP.

### 1.1.2. Treatment Assignment, Database Lock and Unblinding

#### 1.1.2.1. Randomization

One hundred twenty (120) healthy subjects were to be randomized to vaccination or matching placebo (2:1). Eligible subjects were randomized to receive one administration of Sing2016 M2SR H3N2 influenza vaccine or saline placebo on Study Day 1. The randomization was a permuted block of size 3. Randomization was local to each site. The randomization was stratified on pre-screening antibody to HAI (<40 versus ≥40 to ≤320) obtained under a separate, site-specific ICF.

#### 1.1.2.2. Database Lock

The clinical database will be locked after the last subject has completed visit 05/Day 29 QIV or has terminated the trial early and decisions regarding exclusion of individual subject data is documented following the Data Review meeting.

#### 1.1.2.3. Treatment Blinding

ClinDart Data Management (DM) is responsible for supporting ongoing blinded data review and tracking throughout the study. DM will have access to treatment codes only after the database is locked. North Rim Consulting Statistician and programming staff will have access to treatment codes throughout the trial and will be responsible for managing information so as not to unblind other team members.

## 2. ANALYSIS POPULATIONS

Five populations will be formed: Randomized Set, two Safety Sets, and two Evaluable Sets. If necessary two additional per protocol populations will be formed.

#### 2.1.1.1. Randomized Set (RS) Population

The RS consists of all subjects randomized to the study. Results for this set will be reported by randomized treatment.

#### 2.1.1.2. IP Safety Set (IPSS) Population

This SS Population will consist of all randomized subjects who receive any IP administration. Safety results will be presented by actual treatment received.

#### 2.1.1.3. Safety Set (QIVSS) Population

This SS Population will consist of all randomized subjects who receive any QIV administration. Safety results will be presented by actual treatment received.

#### 2.1.1.4. IP Evaluable Set (IPEVAL) Population

The IP Evaluable Set will consist of all randomized subjects who receive an IP administration, have an IP Day 29 visit with sample collection, and have no major protocol violations/deviations thought to interfere with evaluation of IP immunogenicity. Results for this set will be reported by randomized treatment. If more than 5% of this population is excluded due to major protocol deviations, then a new set (IPPPEVAL) will be formed, and analyses repeated using this population.

#### 2.1.1.5. QIV Evaluable Set (QIVEVAL) Population

The QIV Evaluable Set will consist of all randomized subjects who receive an IP dose administration, receive QIV, have a QIV Day 29 visit with sample collection, and no major protocol violations or deviations thought to interfere with evaluation of immunogenicity. Results for this set will be reported by randomized treatment. If more than 5% of this population is excluded due to major protocol deviations, then a new set (QIVPPEVAL) will be formed, and analyses repeated using this population.

## 3. SUBJECT DISPOSITION (RS)

N(%) of Subjects will be accounted for as follows:

1. Randomized
2. Received IP administration (IPSS)
3. Received IP administration and had IP Day 29 visit with sampling
4. Received QIV administration (QIVSS)
5. Received IP dose and QIV administration and QIV Day 29 visit with sampling.

Reasons for exclusions from safety and evaluable sets will be

listed.Screen failures were not collected in the database.

## 4. PROTOCOL DEVIATIONS

N(%) of protocol deviations will be displayed by treatment and deviation type (missed visit, prohibited medication, missed procedure, procedure out of window, etc.). Major protocol violations and deviations will be reported in the clinical study report (CSR).

Before database lock, clinical operations, data management, medical and statistical staff will meet and agree on major protocol violations and deviations. Major deviations will exclude subjects from study populations at the Data Review meeting. See FluGen Protocol Deviation Handling Plan, Version 1.0 27 July 2021.

## 5. DEMOGRAPHIC AND BASELINE CHARACTERISTICS (IPSS)

N (%) of subject will be displayed by treatment for gender, race, age, ethnicity, height, weight, BMI, Investigational Site, baseline HAI from FluGen (<10, 10 - <40, >=40), and stratification HAI (<40, 40 - <=320). Summary statistics (N, Mean, SD, Median, Min,Max) will be displayed for continuous variables. N(%) will be displayed for categorical variables.

A second baseline will be necessary (QIV Baseline) to compute change from QIV baseline for vital signs, and immunogenicity.

## 6. MEDICAL HISTORY

Significant medical histories will be listed with condition and start and end dates.Medical histories with no end date will be reported as ongoing. There will be no summary of medical histories.

# 7. CONCOMITANT MEDICATIONS/THERAPIES (IPSS)

Medications will be collected beginning at informed consent, coded according to WHODrug Sep2019 and presented by therapeutic indication (ATC2) and preferred name. Concomitant medications will include all those taken during the study (Day 1 –Day 29 QIV). Medications starting after informed consent and stopped prior to IP dosing will be included in the listings, but not included in the concomitant medications summary. Prohibited concomitant medications/therapies will not be flagged in these tables or listings. Prohibited medications/therapies will be appear under protocol deviations.

# 8. EFFICACY (ANALYSIS

## 8.1. Primary

Efficacy for this study is immunogenicity which is a secondary objective. The primary objective of this study is safety and tolerability.

## 8.2. Secondary

Immunogenicity will be assessed by measuring serum antibody responses by MN and/or HAI assay and mucosal antibody titers by ELISA. Additional immune parameters may be assessed including T cell responses. Samples are collected pre dose, at Day 29, and Days 1 and 29 QIV. Sample collection dates/times will be recorded in the clinical database.

Immunogenicity analyses will be analyzed as randomized. Additional details for specific immunogenicity assays may be added to this SAP as immunogenicity analyses are finalized.

Serum antibody response (influenza-specific HAI, MN, NAI, and ELISA results) will be summarized by treatment comparisons of GMT, GMFR, seroconversion, and seroprotection.

GMTs and GMFRs are displayed below by visit. Note that for approximately 40% of subjects, beginning at the end of September 2021, IP Day 29 and QIV Day 1 sample collection was on the same day.

Geometric Mean Titers and Mean Folds by Visit

| **Sample Collection** | **GMT** | **GMFR – reference** |
|---|---|---|
| IP Day 1 | X | |
| IP Day 29 | X | IP Day 1 |
| QIV Day 1 | X | IP Day 1 |
| QIV Day 29 | X | QIV Day 1 & IP Day 1 |

Ln-transformed GMT and the ln-transformed GMFR ratios will be analyzed using t-tests. In case of baseline imbalance between treatments, analyses may be repeated using a general linear model adjusting for baseline titers.

Conversion rates categorized as >=2-fold and >= 4-fold will be reported for IP Day 29 and QIV Day 29. Treatment comparisons of conversion counts (%) will be done using Fishers Exact Tests. In case of baseline imbalance between treatments, analyses may be repeated using a logistic regression adjusting for baseline titers.

Seroprotection defined as HAI titer >=40 will be reported for IP Day 29 and QIV Day 29. Treatment comparisons of seroprotection counts (%) will be done using Fishers Exact Tests. In case of baseline imbalance between treatments, analyses may be repeated using a logistic regression adjusting for baseline titers.

Summary statistics for IgA response to influenza HA antigens are presented uncorrected and corrected for total IgA.

For the uncorrected analysis all samples are included regardless of total IgA(ug/mL).

For IgA corrected analysis

- If Total_IgA >= 1 then corrected value = Uncorrected/Total_IgA.
- If Total_IgA < 1 then set the corrected value to missing.

For example: SingC = Sing / Total_IgA >=1; Else SingC= missing.

Summaries are GMTs, GMFRs, and N(%) of subjects with >= 2-fold and > = 4-fold rise in mucosal IgA. Summary statistics for secretory IgA immune response (influenza A-specific and total IgA ELISA) at IP Day1, IP Day 29, and QIV Day 29 will be provided.

Depending upon the results of primary and secondary analyses, some or all the following exploratory analyses may not be done. Cell-mediated immunity (CMI) assessed by frequencies and fold-increases of antigen-specific T-lymphocytes may be summarized. Additional influenza-specific assays may be performed, and results summarized. Results of exploratory analyses may be reported separately from the CSR.

### T Cell Mediated Immunity (Cytokines: IFNg, GrzB, IFNg+GrzB)

T Cell-mediated immunity (CMI) results are available for cells that secrete IFNg only, GrzB only, and cells that secrete both IFNg and GrzB. These double-secreting cells (IFNg+GrzB) will be a subset of cells that secret IFNg or GrzB only. CMI results are reported in Spot Forming Colonies (SFC) per 400,000 cells.
Summaries are GMTs, GMFRs, and N(%) of subjects with >= 2-fold and > = 4-fold rise in CMI. Summary statistics for CMI at IP Day1 and IP Day 29 will be provided.

## 8.3. Adjustments for Covariates

No adjustments for covariates beyond potential inclusion of a baseline (IP baseline and QIV

baseline) for immunogenicity are planned.

### 8.4. Handling of Dropouts or Missing Data

Subjects who are withdrawn from the study will not be replaced. Missing data will be considered missing at random and no missing data will be imputed.

### 8.5. Interim Analyses and Data Monitoring

Safety data were reviewed by the safety review committee after Cohorts 1 and 2. There were no additional planned interim reviews or analyses.

Top line results (grouped by treatment) with no subject-level information were permitted by protocol. Any interim review will be reported in the CSR.

### 8.6. Multiple Comparisons / Multiplicity

There are no formal tests of hypotheses thus no adjustment for multiple tests.All p-values are descriptive only.

### 8.7. Examination of Subgroups

Due to the limited size of this study, there are no planned subgroup analyses. Exploratory analyses may examine primary or secondary endpoint results by age groupings, baseline titers or by QIV response, or those receiving Covid vaccine during the study.

## 9. PRIMARY OBJECTIVE: SAFETY EVALUATION (IPSS)

### 9.1. Extent of Exposure

IP exposure will be characterized as recorded on the eCRF. The protocol references actual dose, percentage of expected dose, and percentage of subjects receiving expected dose. The protocol also references vial weights. The eCRF captured only whether complete dose was administered. IP exposure will be reported as IP administered.

### 9.2. Adverse Events (AEs)

Adverse events were to be recorded from the day of informed consent through QIV Day 29. For subjects without QIV administration, follow-up for both AEs and SAEs was 28 days after IP. During the study treatment period subjectswere asked non-leading questions to determine the occurrence of spontaneously reported (unsolicited) AEs.

Symptoms were collected for the 7 days following IP administration using eDiaries, with clinical interpretation of data entries by the site investigator. At the discretion of the investigator, a symptom recorded by the subject on the eDiary may have been reported as an AE. The investigator was responsible for determining the severity and the relationship to IP for any eDiary-based AEs. There was no reconciliation of the database between the eDiary symptoms and other AE reports.

PE findings recorded as clinically significant, severe, or life threatening were to be recorded as AEs. Investigators usedtheir judgement regarding reporting of additional PE findings as AEs.

All AEs will be included in the listings. Only treatment emergent AEs will be summarized.

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

Treatment-emergent AEs (TEAE) are AEs with start dates between administration of theIP and IP Day29. If there is no administration of QIV then AE/SAE follow-up will be 28 days post IP administration. However, AEs with start dates past Day 29, if judged by the investigator to be IP-related, will be included in the treatment emergent set.

All AEs will be coded based on MedDRA (V24.0).

Where provided in Protocol Appendix 1, AEs will be graded according to the Vital Sign and Systemic Toxicity Assessments.

N (%) of subjects with at least one TEAE and N (%) of subjects with at least one treatment-related treatment-emergent AE (TRAE) will be summarized. TEAE and TRAE by worst severity will be summarized.

N (%) of subjects with TEAE will be reported by System Organ Class and Preferred Term (SOC/PT) and maximum severity. N (%) of subjects with TRAE will be reported by SOC/PT and maximum severity.

N (%) of subjects with TEAE by SOC/PT by overall frequency (decreasing) will be reported.N (%) of subjects with TRAE will be reported by SOC/PT by overall frequency (decreasing).

AEs will be reported by start date as: a) following IP administration, b) after IP administration and before QIV administration, c) within 7 days of IP administration, and d) after QIV administration.

No statistical test comparing adverse event rates will be done.

### 9.2.1. Reactogenicity Assessments: eDiaries

Temperature (degrees celsius or fahrenheit to one decimal point), 7 systemic reactogenicity symptoms and 2 local reactions were collected pre-dose and once daily for the 7 days following IP administration via a reactogenicity eDiary, see Protocol Appendix 1. Symptoms were reported by the subject, at 5 levels of intensity (0=None, 1=Mild, 2=Moderate, 3=Severe, 4= Medically attended).

Investigators (or designee) were required to review the reactogenicity eDiary data daily as part of the ongoing safety review. The site was to be notified if a subject had entered a Grade 3/4 severity for a symptom. The site was then required to contact the subject to confirm the scoring and determine whether subject should attend the clinic for further follow-up. The Investigator was required to evaluate the symptoms and assess whether they agree with the grading of 3 or higher. Regardless of the PI assessment, the diary entries were not to be changed, and the Investigators were to either verify the patient-recorded score or note a discrepancy in the source documentation. If any discrepancies or changes in reporting were noted at review, both uncorrected and corrected data were to be recorded and both sets of symptom scores transferred from the eDiary vendor. Corrected eDiary data will be summarized in the CSR. Original data entries as recorded by the patient and/or where these differ from final reports will be given in an appendix to the CSR. Missing eDiary data will be reported as missing; no symptom values were to be imputed.

Daily temperatures and worst (maximum) temperature over the 7 days experienced by each subject will be summarized using descriptive statistics.

Worst symptom severity experienced over the 7 days and the number of days with any grade of symptom (score > 0) will be summarized using frequencies and percentages.

These summaries will use the IPEVAL set.

Symptoms tables include both local reactions and systemic symptoms.

### 9.3. Clinical Laboratory Evaluation

Clinical safety labs are collected pre-IP administration. There are no scheduled safety lab collections after IP administration except as ordered/requested by the investigator or safety/medical monitor for specific diagnostic or AE follow-up. Any laboratory-based AEs will be reported based on the Grading Scale for Laboratory AEs – Modified from FDA GradingScale as Appropriate for Local Lab (Protocol Appendix 4).

A listing of clinical laboratory test results (screening and as-requested) will be provided. Results will be flagged as outside reference ranges and, where appropriate, graded according to the FDA Grading Scale (see protocol Appendix 3 & 4). Clinically significant (CS) values or CS changes from baseline values were to be recorded as AEs.

### 9.4. Vital Signs

Blood pressures, heart rate, respiratory rate and temperature will be summarized at each time point by descriptive statistics for the result and for the change from baseline (IP and then QIV baseline).CS values or CS change from Baseline were recorded as AEs at the discretion of theclinical investigator.

Vital signs were graded according to the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2008, and noted as clinically significant. Vital signs to be assessed include: fever, tachycardia, bradycardia, systolic and diastolic hypertension, diastolic hypotension, and respiratory rate. These VS will be reported by shift tables of worst grade recorded post IP administration through QIV Day 29.

### 9.5. Physical Examinations

A complete physical exam to determine the subject's suitability for the study was done prior to IP administration. Limited physical examinations consisting of nasal, lung and throat assessments were done predose, and at 15 minutes post dose on IP Days 1, 8, and 29. A dermal injection site assessment was done on QIV Days 1 & 29. At the discretion of the Investigator, physical examination may have been performed on other body systems. Clinically significant findings may be recorded as AEs. Physical examination findings (CS and NCS) will be included in the listings.

### 9.6. Halting Rules (Protocol Section 7.3)

Events meeting the halting rule criteria will be listed by subject and treatment.

## 10. HYPOTHESES TO BE TESTED

No formal hypotheses are to be tested. P-values for immunogenicity are descriptive only.

## 11. DETERMINATION OF SAMPLE SIZE

The sample size of 80 subjects per active IP group and 40 for placebo was chosen based on reports of studies of othervaccines in early-stage development. This study is not powered for a formal comparison of IP to placebo.

## 12. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNEDANALYSES

Unavailability of some pre-dose Day 1 samples made it necessary to replace these samples with Screening samples.

Screening results used to stratify treatment assignment were replaced in data presentations by HAI results generated at Flugen.

Descriptive p-values for within-group changes (GMFR) were added to displays.

Antigen specific T cell values were completed at Flugen and levels reported to NRC for analyses. Documentation of the pre-processing of samples are documented at Flugen.

E-diary data collection and transfer initially proved problematic. Issues were resolved and data transfers corrected by the vendor. Corrected e-diary data will be presented in the CSR. Original values will be listed in an appendix.