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Statistical Analysis Plan

Phase 1b clinical study to investigate the safety and immunogenicity of the Bris10 (A/Brisbane/10/2007) M2SR and Sing2016 (A/Singapore/INFIMH-16-0019/2016) M2SR H3N2 monovalent influenza vaccines

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Signature Page

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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.

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-V003 dated 18-May-2020 (v8.0).
2. All 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; Day 29 is the 2nd IP administration.
4. Baseline (IP Baseline) is the last observation prior to IP administration, at the data point, 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. This study is not powered to detect differences in safety or immunogenicity between active and placebo cohorts. The sample size of 250 (200 active subjects and 50 placebo subjects) was chosen empirically based on studies of other vaccines in early stage development.
6. There is no plan for test of hypotheses; p-values generated are descriptive only.
7. Where possible raw data (not results from calculations) are transferred from external vendors.

1.1.2. Treatment Assignment, Database Lock and Unblinding

1.1.2.1. Randomization

Two hundred-fifty (250) subjects were to be randomized to vaccination or matching placebo (1:1:1:1:1). Eligible subjects were randomized to receive two administrations (Days 1 and 29) of Sing2016 at three dose levels, Bris10 at one dose level, or placebo The randomization was a permuted block of size 5 with early blocks (sentinel groups) weighted to lower doses of Sing2016. Later blocks were balanced across the 5 treatments (expansion groups). Randomization was local to each site. The randomization was not stratified on any subject baseline characteristics.

1.1.2.2. Database Lock

The database will be locked after the last subject has had the day 209 visit (or terminated the trial early). The Day 209 visit may be by telephone.

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 provide SDTM datasets to NRC Statistical Programming. Statistician (Roger Aitchison) and his designees will have

access to treatment codes throughout the trial and will be responsible for managing data so as not to unblind other team members.

2. ANALYSIS POPULATIONS

Three populations will be formed: Randomized Set, Safety Set, and the Evaluable Set.

2.1.1.1. Randomized Set (RS) Population

The RS consists of all subjects randomized to the study.

2.1.1.2. Safety Set (SS) Population

The SS Population will consist of all randomized subjects who receive at least one IP administration.

2.1.1.3. Evaluable Set (EVAL) Population

The Evaluable Set will consist of all randomized subjects who receive both administrations of IP.

3. SUBJECT DISPOSITION (RS)

Subjects will be accounted for as follows:

1. N(%) of subjects randomized
2. N(%) of subjects who received first IP administration (SS)
3. N(%) of subjects who received second IP administration (EVAL)
4. N(%) of subjects who complete Treatment Period (Day 57)
5. N(%) of subjects who complete Follow-up (Day 209).

Reason for exclusion from safety and evaluable sets will be tabulated.

Screen failures were not collected in the database.

4. PROTOCOL DEVIATIONS (SS)

N(%) of protocol deviations will be displayed by treatment and deviation type (missed visit, prohibited medication, missed procedure, procedure out of window, etc.). Protocol deviations resulting in subject exclusion from the evaluable set will be reported in the clinical study report (CSR).

5. DEMOGRAPHIC AND BASELINE CHARACTERISTICS (SS)

N (%) of subject will be displayed by treatment for gender, race, age, ethnicity, height, weight, BMI, and Investigational Site. Summary statistics (N, Mean, SD, Median, Min, Max) will be displayed for continuous variables. N(%) will be displayed for categorical variables.

6. MEDICAL HISTORY (SAFETY SET)

Significant medical histories will be listed with condition and start and end dates. Medical histories with no end date will be reported as continuing.

7. CONCOMITANT MEDICATIONS/THERAPIES (SS)

Medications collected from informed consent and will be coded according to Who Drug (WHODrug Sep2019) and presented by therapeutic main group (ATC2) and preferred term. Concomitant medications will include all those taken during the study (Day 1 – Day 57). Medications starting after informed consent and stopped prior to Day 1 will be included in the listings, but not in the concomitant medication summary.

8. EFFICACY ANALYSIS

8.1. Primary

The primary objective of this study is safety. Efficacy is not a primary objective.

8.2. Secondary

Immunogenicity data sources include influenza-specific serum antibody responses (MN, HAI, ELLA) and mucosal IgA antibody responses as listed below.

External Vendor-supplied data to be integrated into the clinical database.

Vendor Name	Specimen Material Type	ISTESTCD (Test Virus)	ISSCAT (Assay Type)	Comment
Southern Research	SERUM	BRIS	MN	No LLOQ, lowest values reported = 5.
Southern Research	SERUM	SING	MN	No LLOQ, lowest values reported = 5.
Viroclinics	SERUM	BELG	MN	
Viroclinics	SERUM	BRIS	HAI	

Viroclinics	SERUM	H6N2	ELLA	
Viroclinics	SERUM	KANS	HAI	.
Viroclinics	SERUM	KANS	MN	
Viroclinics	SERUM	Hong Kong	HAI	
Viroclinics	SERUM	Hong Kong	MN	
Viroclinics	SERUM	SING	HAI	
Viroclinics	SERUM	SWTZ	HAI	
Viroclinics	SERUM	SWTZ	MN	
VisMederi	NASAL MUCUS	BRIS	SIgA	LLOQ=3 ; set to 1.5. At the replicate level.
VisMederi	NASAL MUCUS	SING	SIgA	LLOQ=3 ; set to 1.5. At the replicate level.
VisMederi	NASAL MUCUS	TOTIGA	SIgA	No LLOQ.
VisMederi	NASAL MUCUS	BRISC= BRIS/TOTIGA	SIgA	BRIS to be corrected for TOTIGA. If TOTIGA <1 then BRISC= missing, otherwise BRISC=BRIS/TOTALIGA.
VisMederi	NASAL MUCUS	SINGC= SING/TOTIGA	SIgA	SING to be corrected for TOTIGA. If TOTIGA <1 then SINGC= missing, otherwise SINGC=SING/TOTIGA

Serum antibody response (influenza-specific HAI, MN, and ELLA results) after one and after both administrations of M2SR vaccine will be summarized by treatment comparisons of GMT, GMFR, seroprotection, and seroconversion (2-fold and 4-fold).

GMTs will be summarized at Days 1, 29, and 57; GMFR will be summarized by Day 29/Day 1, Day 57/Day 1, and Day 57/Day 29. Ln-transformed GMT and the ln-transformed GMFR ratios will be analyzed using ANCOVA with baseline (Day 1) values as a covariate. [Note: GMFR Day 57/Day29 is modelled with Day 1 baseline covariate.]

Conversion rates categorized as >=2-fold and >= 4-fold values will be reported for Days 29 and 57. Pairwise comparisons of conversion counts (%) will be done using Fishers Exact Tests.

Seroprotection rates at Days 1, 29 and 57 will be presented for the hemagglutination inhibition (HAI) assay only. Pairwise comparisons of seroprotection counts (%) will be done using Fishers Exact Tests.

8.2.1. Secretory IgA Immune Response and Viral Shedding from Nasal Swab

Summary statistics for secretory IgA immune response (influenza A-specific and total IgA ELISA) at Days 1 (baseline), 29, and 57 post IP administration will be provided.

Summary statistics for viral shedding (influenza A-specific q-PCR) at Screening, Days 1 and 8, will be provided.

8.3. Exploratory

Depending upon the results of primary and secondary analyses, some or all 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.

8.4. Adjustments for Covariates

No adjustments for covariates beyond baseline values are planned.

8.5. Handling of Dropouts or Missing Data

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

No missing data will be imputed.

8.6. Interim Analyses and Data Monitoring

There are no planned interim analyses.

Top line results (grouped by treatment) with no subject-level information may be produced prior to data base lock for sponsor review.

8.7. Multiple Comparisons / Multiplicity

There are no formal tests of hypotheses thus no adjustment for multiple tests.

All p-values are descriptive only.

8.8. Examination of Subgroups

There are no planned subgroup analyses.

9. PRIMARY OBJECTIVE: SAFETY EVALUATION (SS)

9.1. Extent of Exposure

IP exposure will be characterized by the number of IP administrations received (1 or 2) and N (%) of subjects reported as receiving a complete dose as recorded on the eCRF. Summary statistics will be provided for change in delivery device weights to reflect amount of IP delivered. IP exposure tables will display results by the 5 treatment groups and will be repeated for the SS and the EVAL sets.

9.2. Adverse Events (AEs)

Adverse events were to be recorded from informed consent through Day 57 + 28 days. For subjects receiving only one IP administration, follow-up was 28 days. Serious adverse events were recorded through Day 209. During the study treatment period (through Day 57) subjects were asked non-leading questions to determine the occurrence of spontaneously reported (unsolicited) AEs.

Symptoms were collected for the 7 days following each IP administration using Symptom Memory Aids (SMA), with clinical interpretation by the site investigators. A symptom recorded by the subject on the Symptom Memory Aid (SMA) may or may not have been reported as a solicited AE. For clinical interpretation Investigators used the, *"Instructions to site investigators on assessing AEs from symptom memory aids (16 October 2019)"* as a guideline in assessing whether a symptom was to be reported as an AE. The investigator was responsible for determining the severity and the relationship to IP for these SMA-based (solicited) AEs. There was no reconciliation of the database between the SMA symptoms and adverse event reports.

Symptom Memory Aids were used to facilitate assessment of AEs, as described in Protocol Section 6.2.1.6. Events that were reported in the Symptom Memory Aid were reviewed by the study staff and assessed and recorded as AEs. Investigators were instructed to clinically assess, and record individual symptoms reported by subjects as AEs in order to ensure that all reactogenicity events were captured. However, Protocol Section 9.6 states "Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). After medical review of the AE listing, it appears that some AEs should be grouped as diagnoses. For example, a subject who reported concurrent cough, runny nose, and sneezing assessed by the investigator as unrelated to vaccine should have those symptoms grouped as a syndromic diagnosis (URI), with the severity based on the most severe

AE/symptom. Therefore, as a secondary presentation, subjects with 3 or more concurrent unrelated AEs of cough, runny nose/rhinorrhea, sneezing, sore throat, itchy throat, itchy eyes, congestion, nasal irritation, hoarseness, headache, body aches/myalgia, tiredness/fatigue, malaise, and fever will have those AEs grouped as URI. This will simplify presentation of the data and is consistent with the protocol.

To facilitate the above reporting standard a total of 15 different AE Preferred Terms (Arthralgia, Cough, Fatigue, Headache, Malaise, Myalgia, Nasal congestion, Nasal discomfort, Nasopharyngitis, Oropharyngeal pain, Paranasal sinus discomfort, Pyrexia, Rhinorrhea, Sinus congestion, or Throat irritation) were considered for contribution to a syndromic diagnosis of URI. Subjects who reported symptoms that resulted in coding of 3 or more of these PTs on a given day, assessed by the Investigator as unrelated to the vaccine, were reported as URI. The severity of the URI was reported as the worst severity of the qualifying AEs considering only days when 3 or more terms were reported. AEs already reported by the Investigator as a URTI were not included in the URI data display.

Targeted physical examinations were conducted throughout the study. PE findings recorded as clinically significant, severe, or life threatening were recorded as AEs. Investigators used their judgement regarding reporting of additional PE findings as AEs.

Clinical laboratory results marked as clinically significant by the Investigator were captured as AEs.

AE's with start dates prior to the first IP administration will be included in the listings, but not in the summaries.

Treatment-emergent AEs (TEAE) are AEs with start dates between administration of the first IP and last IP administration + 30 days. However, if an AE is related to IP then there is no upper bound on the start date. Thus, treatment related TEAEs may have start dates beyond 30 days post IP administration.

All AEs will be coded based on MedDRA (V20.0).

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. Note that the protocol uses the term "vaccine associated" AEs, but the eCRF collects AEs as related, not related.

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).

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 a serious AE, if any, will be provided.

No statistical test comparing adverse event rates will be done.

9.2.1. Reactogenicity Assessments: Symptom Memory Aide (SMA)

Fourteen (14) different reactogenicity symptoms (including other/specify) are collected pre-IP administration and once daily for the 7 days following each of the IP administrations via a "Symptom Memory Aide (SMA)", see Protocol Appendix 5. Symptoms are reported by the subject, at 4 levels of intensity (0=None, 1=Mild, 2=Moderate, 3=Severe).

Each symptom will be summarized by severity (worst reported over the course of the 7 days) by treatment and whether subjects experienced a given symptom at any severity. This summary will be done for IP administration 1 and repeated for IP administration 2. Symptom category "other" will be included in the listings only.

Each symptom will be summarized by the number of days with symptoms for each subject. This summary will be done for IP administration 1 and repeated for IP administration 2. Symptom category "other" will be included in the listings only.

Each symptom (and oral temperature) will be summarized by IP administration (1 or 2) and timepoint (Day 1 pre-dose (coded as Day 0), Day 1 post dose, Days 2-7) . Summary statistics will be given for oral temperature (mean, SD, median, min, max) and as yes/no (0,1, missing) for each symptom. Symptom category "other" will be included in the listings only.

These summaries will use the EVAL set.

SMA symptoms will not be grouped into local and/or systemic events.

9.3. Clinical Laboratory Evaluation

A central clinical laboratory will be used for this study. Laboratory-based AEs will be reported based on the Grading Scale for Laboratory AEs – Modified from FDA Grading Scale as Appropriate for Local Lab (Protocol Appendix 2).

Summary statistics of test results and change from Baseline test results will be done for all serum chemistries and hematology.

For serum chemistries and hematology, and urinary assessments where FDA vaccine grading scale is applicable (Protocol Appendix 2), maximum shift from Baseline in severity will be provided.

Urinalysis (dipstick) results will be listed and not summarized.

A listing of clinical laboratory test results outside the reference ranges will be provided.

Clinically significant (CS) values or CS change from baseline values were to be recorded as AEs at the discretion of the clinical and safety monitor.

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

CS values or CS change from Baseline were recorded as AEs at the discretion of the clinical investigator.

9.5. Limited and Targeted Physical Examinations

A 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 5 and 30 minutes post dose on Days 1 and 29, and at Days 8, 36, 57 and 209. At the discretion of the Investigator, targeted physical examination may have been performed on other body systems and findings may be recorded as AEs. Physical examination findings 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 are descriptive only.

11. DETERMINATION OF SAMPLE SIZE

The sample size of 50 subjects per group was chosen based on reports of studies of other vaccines in early stage development. This study is not powered for any formal treatment comparisons.

12. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

The study was planned to enroll 50 subjects per treatment group. Enrollment was halted at approximately 40 subjects per group due to seasonal flu considerations.

Due to COVID-19 some Day 209 follow-up visits were conducted by telephone.

Secondary and exploratory endpoint data for CMI (ELISPOT) and viral shedding (influenza A-specific q-PCR) data were not available for analysis at database lock.

These and additional exploratory study endpoints may be the subject of future analyses.