CM4620-205 Statistical Analysis Plan

A Single-Blind Dose-Ranging Pharmacodynamic Study of Auxora for the Treatment of Patients with Critical COVID-19 Pneumonia

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STATISTICAL DESIGN AND POWER

INTRODUCTION
This document outlines the proposed analyses for the Auxora trial. This phase II, single center, single-blind, randomized controlled trial is designed to assess the pharmacodynamic response of of bronchoalveolar lavage (BAL) T cell/monocyte subsets and chemokine release to various doses of Auxora in patients with critical COVID-19 pneumonia. The purpose of this document is to provide detail regarding the statistical analysis plan (SAP) for this study. Northwestern University Data Analysis and Coordinating Center (NUDACC) will spearhead overall SAP development and maintain all versions. NUDACC will also implement these analyses as specified, and will ensure appropriate inference and interpretation in dissemination materials.

STUDY OBJECTIVES
Primary
- To assess the pharmacodynamic response of bronchoalveolar lavage (BAL) T cell/monocyte subsets and chemokine release to various doses of Auxora in patients with critical COVID-19 pneumonia

Secondary
- To assess the safety and tolerability of Auxora in patients with critical COVID-19 pneumonia
- To assess the serum and BAL pharmacokinetic profile of various doses of Auxora in patients with critical Covid-19 pneumonia

STUDY METHODS
Trial Design
This will be a single center, prospective randomized trial of patients ≥ 18 years of age critical SARS-CoV-2 infection. The patient will not know if they are receiving Auxora or Placebo. The first 4 patients will be enrolled in Cohort 1 and randomized 3:1 to Auxora or Placebo. If dose escalation occurs, the next 8 patients will be enrolled in Cohort 2 and randomized 3:1 to Auxora or Placebo. If dose escalation continues, the next 8 patients will be enrolled in Cohort 3 and randomized 3:1 to Auxora or Placebo. The decision to escalate dosing will be made by CalciMedica in consultation with the PI after the review of safety events in Cohorts 1 and 2.

Randomization
Patients will be prospectively randomized after obtaining informed consent and confirmation of study eligibility. Patients will be randomized 3:1 via permuted block randomization, with blocks of varying sizes, stratified by cohort. The randomization sequence will be created by the Northwestern University Data Analysis and Coordinating Center (NUDACC), serving as the Data Coordinating Center (DCC) and uploaded to the REDCap database. Once a patient's eligibility has been confirmed, the research coordinator will access the randomization assignment via REDCap. The patient will be randomized and the assignment will be communicated to the pharmacy.

Interim Analysis and Stopping Guidelines
As this is a dose-escalation study, Consultation between the PI and CalciMedica will occur if any patient receiving Auxora in Cohorts 1, 2 or 3 experiences one of the cardiac conduction events listed below during the 144 hours after the Start of the First Infusion of Study Drug (SFISD):
- QTcF interval of ≥ 500 msec; or
- QTcF prolongation of ≥ 60 msec as compared to baseline; or
- Mobitz Type II second degree atrioventricular (AV) block; or
- Third degree or high grade AV block; or
- Polymorphic Ventricular Tachycardia;

If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 1 but the event is not thought to be dose limiting, 4 additional patients will be enrolled in Cohort 1 and randomized 3:1 to Auxora versus Placebo for a total of 8 patients enrolled in the cohort, 6 randomized to Auxora and 2 to Placebo. The PI

and CalciMedica will consult after all 8 patients are enrolled to determine if dose escalation to Cohort 2 will occur. If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 1 and the event is dose limiting, further dosing will not occur using the planned dose regimens but may occur using a regimen that provides lower total exposure of drug.

If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 2 but the event is not thought to be dose limiting, 4 additional patients will be enrolled in Cohort 2 and randomized 3:1 to Auxora versus Placebo for a total of 12 patients enrolled in the cohort, 9 randomized to Auxora and 3 to Placebo. The PI and CalciMedica will consult after all 12 patients are enrolled to determine if dose escalation to Cohort 3 will occur. If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 2 and the event is dose limiting, further dosing will not occur in either Cohorts 2 or 3. 4 additional patients may be enrolled in Cohort 1 and randomized 3:1 to Auxora versus Placebo.

If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 3 but the event is not felt to be dose limiting, 4 additional patients will be enrolled in Cohort 3 and randomized 3:1 to Auxora versus Placebo for a total of 12 patients enrolled in the cohort, 9 randomized to Auxora and 3 to Placebo. If one of the listed safety events occurs in a patient who is receiving Auxora in Cohort 3 and the event is dose limiting, further dosing will not occur in Cohort 3. 4 additional patients may be enrolled in Cohort 2 and randomized 3:1 to Auxora versus Placebo.

There will be no formal interim analyses for efficacy. In addition to the protocol specified safety events specified above, Adverse Events (AEs) and Serious Adverse Events (SAEs) will be recorded on dedicated CRFs and will be reviewed on an ongoing basis by the DSMB.

The sponsor, CalciMedica, reserves the right to terminate the study at any time or for any reason including:

- A directive from the FDA
- A lack of enrollment
- A recommendation from the IDMC

STATISTICAL PRINCIPLES

Level of significance

All analyses will assume a two-sided type I error rate of 0.05.

Analysis population

Pharmacodynamic and Efficacy analyses will be based on an Intention-to-treat (ITT) principle. All data will be included and no subjects will be excluded because of protocol violations. Safety analyses will be based on a per-protocol principle, whereby subjects will be included in the treatment group according to the treatment they actually receive.

Power Considerations

Sample size considerations were based on precision estimates and the ability to construct 95% confidence intervals around point estimates for pharmacodynamics endpoints with reasonable precision. Sample sizes as small as 9 patients per arm will allow for construction of 95% confidence intervals with half-widths as small as 0.77 standard deviation units.

For other assessments, the study is not powered for analysis with inferential statistics.

OUTCOMES AND COVARIATES

Primary Pharmacodynamic Endpoint

- Decrease in combined CD4, CD8, and monocyte proportions in BAL fluid

Secondary Pharmacodynamic Outcomes

- Change in the individual proportions of immune cell in BAL fluid:
    - Tregs
    - CD4 T

- CD8 T
- B cells
- NK cells
- Macrophages
- Monocytes
- Neutrophils

Efficacy Endpoints

- All-cause mortality at Day 60
- Number of days on mechanical ventilation after randomization
- Number of Days in the Hospital after randomization
- Number of Days in the ICU after randomization

Safety Endpoints

- Pre-defined changes in cardiac conduction
- The incidence of TEAEs and SAEs
- The intensity and relationship of TEAEs and SAEs
- Changes in serial SOFA scores
- Mortality at Day 30

Baseline Patient Characteristics

Patient demographic and baseline characteristics will be summarized by mean, standard deviation, median, minimum, and maximum for continuous variables; and by counts and percentages for categorical variables. There will be no formal hypothesis testing for comparison of baseline characteristics between treatment arms.

STATISTICAL CONSIDERATIONS

Methods to assess assumptions

Histograms and boxplots will be used to graphically assess normality of the outcomes. Residual diagnostics will be examined and relevant assumptions will be assessed using graphical display and statistical testing where appropriate. In situations where modeling assumptions are in question, nonparametric methods, transformations, or inclusion of higher order terms, may be applied.

Missing data

Every effort will be made to minimize missing data. Field validations will be programmed into the REDCap project, with fields marked as 'required' as appropriate, to prevent missing data. Data quality rules within REDCap will allow for checks for missing data, and data status and quality reports throughout the duration of the trial will be reviewed on an ongoing basis. If missing values are identified, the DCC will contact the site study coordinator to resolve all issues. The REDCap Data Resolution Workflow will be used to monitor resolution of all data queries and the project coordinator will confirm responsiveness by the study teams. Before performing any final analyses, the database will be assessed for any remaining missing data. Frequency and reasons for missing data will be reported, as available.

STATISTICAL ANALYSES

Pharmacodynamic Analysis

Primary analyses will leverage descriptive statistics to summarize the change in combined proportions of CD4, CD8, and monocytes in BAL fluid by dose arm. Point estimates and corresponding 95% confidence intervals will be reported. We will explore ANCOVA models to compare the difference in post-dose proportions by arm, adjusting for pre-dose levels. Normality assumptions will be assessed and in the event of violation of assumptions, transformations or nonparametric approaches will be considered. Subsequent pairwise comparisons of each dose arm to placebo arm will be assessed as appropriate. Similar methods will be employed for additional flow cytometry endpoints as described above.

Exploratory analyses may also consider unsupervised methods including hierarchical clustering to further identify and visualize patterns in flow cytometry data.

Following single-cell RNA-sequencing, variable genes will be identified using a likelihood-ratio test in DESeq2. K-means clustering will then be used with selected genes to identify homogenous clusters with unique gene expression.

Efficacy Analysis

The date of each hospital admission and discharge will be collected for each subject. The number of days in the hospital after randomization will be summarized by treatment group. We will explore nonparametric comparisons of days in hospital after randomization between arms. Similar analyses will be employed for number of days on mechanical ventilation after randomization and number of days in the ICU after randomization.

Safety Analysis

Mortality rate at Day 30 for each treatment group will be estimated by the Kaplan-Meier procedure.
Safety variables will be tabulated by treatment groups and presented for all treatment patients. Exposure to study treatments, reasons for discontinuation, deaths and causes of deaths will be tabulated. Treatment-emergent AEs (TEAEs) are defined as events that first occurred or worsened after the first dose of study drug. TEAEs will be mapped to the appropriate System Organ Class (SOC) and Preferred Term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA).

Summaries will be provided for all AEs, AEs considered related to study treatment, SAEs, and related SAEs, AE leading to treatment discontinuation and AE leading to death as follows:

- By maximum severity
- Incidence by SOC (by severity grade and overall)
- Incidence by PT (by severity grade and overall)

Laboratory test results will be used in the summary. The lower and upper reference range values for tests from the laboratories will be used to grade the lab results to low, normal and high Shift tables will display (1) shift from baseline grade to the worst grade, and (2) shift from baseline grade to the last grade.

Vital signs will be summarized by visit using proportion of patients with each vital sign being too high or too low according to conventionally accepted vital sign normal ranges.

Exploratory analyses will also consider a linear mixed effect model to assess changes in serial SOFA scores over time. Fixed effects will include arm, time, and the interaction term; a random subject effect will allow for separation of within person and between person variance components.

Concomitant medication will be coded by the WHO Drug Dictionary and summarized by Therapeutic subgroup and preferred terms, using counts and percentages. Concomitant medications are the medications taken with a start date on or after the start of the administration of study treatment, or those with a start date before the start of the administration of study treatment and a stop date on or after the start of the administration of study treatment.

Descriptive statistics will include mean, standard deviation, minimum, median, and maximum for guadecitabine and decitabine PK concentrations.

Pharmacokinetic Analysis

Refer to protocol for more details. CalciMedica will be responsible for implementing PK analyses.

TECHNICAL DETAILS

The SAP will be subject to version control, and we anticipate modifications to analytic plans to be documented herein. As in any study, the analytic plan may change due to assumption violations, logistical issues, unexpected empirical distributions of study outcomes, or a combination thereof. In these cases, the SAP will be

updated accordingly. All analyses will be performed via SAS version 9.4 or higher (The SAS Institute; Cary, NC) or R version 3.6.0 or higher (The R Foundation for Statistical Computing platform). Table and figure formatting and style may be dictated by mode of dissemination or specific target journal(s) for results dissemination.