Statistical Analysis Plan for the IL-6 Biomarker Analysis of Tocilizumab and Sarilumab

IL-6 Biomarker Analysis SAP Version 1.0 dated 08 January 2024

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1. IL-6 BIOMARKER ANALYSIS SAP VERSION

The version is in this document's header and on the cover page.

1.1. Version history

Version 1: Finalized on 8th January, 2024.

2. SAP AUTHORS

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3. INTRODUCTION

This statistical analysis plan (SAP) is for a biomarker analysis of the tocilizumab/sarilumab interventions in the Immune Modulation Therapy Domain in the pandemic stratum of the REMAP-CAP trial. This biomarker analysis is intended to evaluate whether the efficacy of interleukin-6 receptor antagonists (IL6Ra) varied by baseline interleukin-6 level in a subset of participants enrolled in the Immunoglobulin domain in the United Kingdom that underwent biological sampling. This plan is an appendix to the COVID-19 Immunomodulation Domain SAP version 1.1 dated 15 April 2021. This analysis is intended to be exploratory, and the authors of this document have been unblinded to the results of the primary analysis of the Immune Modulation domain. Although this plan was not prespecified prior to unblinding, it uses the analysis principles outlined in the original domain SAP.

4. INTERVENTIONS

This analysis will include the following interventions in the Immune Modulation Therapy domain:

1. No immune modulation for COVID-19 (control)
2. Tocilizumab (IL-6 receptor antagonist; IL6Ra)
3. Sarilumab (IL-6 receptor antagonist; IL6Ra)

Tocilizumab and sarilumab will be pooled into a “Pooled IL6Ra” intervention for this analysis.

5. DISEASE STATES

There are two disease states in the Pandemic Appendix to the Core Protocol (PAtC), which are **moderate** and **severe**. The IL6Ra interventions were not available in the moderate state, so this analysis will be restricted to the severe state.

6. ANALYSIS POPULATION

The population for this analysis is the **Baseline IL6 Intention to Treat (ITT)** population defined as all patients with available baseline IL-6 values (serum or plasma) that were randomized to tocilizumab, sarilumab, or control and were eligible for tocilizumab or sarilumab.

7. ENDPOINTS

The following end points will be analyzed, displayed graphically, and summarized through descriptive statistics:

1. **Organ Support-Free Days** (OSFD)
    a. An ordinal endpoint with mortality as the worst outcome. The primary endpoint for the REMAP-CAP Pandemic Infection Suspected or Proven (PISOP) stratum. The organ support considered is cardiovascular (vasopressor/inotrope support) and respiratory support. See Appendix A of the Immune Modulation SAP for a detailed description.

2. **In-Hospital Survival**
    a. A dichotomous endpoint of in-hospital death where the death component corresponds to a –1 on the OSFD endpoint.

8. GRAPHICAL DATA SUMMARIES

1. All ordinal endpoints will be plotted using stacked cumulative bar plots and cumulative probability plots.

9. DESCRIPTIVE STATISTICS

1. Ordinal endpoints will be summarized by the cumulative frequency of each outcome. The 25th, 50th, and 75th percentiles will be summarized.
2. Dichotomous endpoints will be summarized by the proportion in each category.

10. ANALYTIC APPROACH

Each inferential analysis will be done using a Bayesian model. A summary of the analyses methods is provided below.

10.1. Primary Analysis of Primary Endpoint

The **primary analysis model** is a Bayesian cumulative logistic model for the ordinal primary endpoint. The primary endpoint for the severe state has 23 possible ordered outcomes. Let the outcome for a patient by labeled as Y_i, with possible values, –1 (death), 0, 1, …, 21. A cumulative logistic model is specified. The model is structured so that an odds-ratio >1 implies clinical benefit. The model has factors for:

- Each level of the ordinal endpoint
- Each global site, nested within country
- Age; ≤39, 40-49, 50-59, 60-69, 70-79, 80+
- Sex

- Time; quarterly (12 week) buckets of time working backwards from the last enrolled patient.
- An effect for each intervention

The analyses will use the following conventions to address potential problems with model stability:

- All sites within a country that have <5 patients randomized will be combined into a single site within that country.
- If there is an outcome in the ordinal scale that did not occur in the data in a given state, then that outcome will be combined with a neighboring outcome (the worse outcome). This is done for model stability. For example, if the outcome 11 never occurred in the severe state, then a combined outcome of 10 & 11 will be modeled for the severe state in that analysis.
- Time buckets with <5 randomized subjects in a state may be combined with the more recent neighboring bucket for that state.

10.2. Analytic Approach for Secondary Dichotomous Endpoints

A Bayesian logistic regression model will be used for each dichotomous outcome. The model will always specify the "event" as the negative outcome and be parameterized so that an odds-ratio >1 implies benefit to patients. The model is the standard logistic link function model:

$$log\left(\frac{\pi}{1-\pi}\right) = \alpha - [factors]$$

References will be made to the factors in the model and their prior distribution. Many of these factors will be the same as the primary analysis model, with the same priors, as the parameters have similar interpretation. For example, all in-hospital mortality models should use the Beta prior distribution implied by the Dirichlet prior in the OSFD model.

10.3. Baseline IL-6 Analyses

Analyses will explore baseline IL-6 as a potential prognostic covariate and effect modifier for IL6Ra interventions. Several different modeling strategies are proposed to explore the relationship of baseline IL-6, outcome, and treatment effect.

First, groups will be defined based on terciles of the baseline IL-6 values. Analyses will be run with baseline IL-6 tercile as a covariate with a single common effect of the pooled IL6Ra interventions estimated with a standard normal prior. Additionally, analyses will be run with IL-6 tercile as a covariate and with subgroups defined based on IL-6 tercile. In this analysis, separate effects of the pooled IL6Ra intervention will be estimated within each tercile group. The treatment effect within each subgroup will be estimated with independent standard normal priors.

A second set of analyses will be performed defining groups of baseline IL-6 based on every cumulative quantile from 5% to 95% and using a smoothing prior on the effect across groups. First, analyses will estimate covariate effects across the 20 subgroups with a first-order normal dynamic linear model (NDLM) prior and a single common effect of the pooled IL6Ra interventions. Second, analyses will estimate covariate effects with an NDLM and separate treatment effects within each subgroup with a NDLM. The NDLM is defined by sequential priors on the effects for each group:

$$\theta_1 \sim N(0,1)$$

$$\theta_t \sim N(\theta_{t-1}, \tau^2); t = 2, \dots, 20$$

Where the "drift" parameter has the following hyperprior

$$\tau^2 \sim IG(0.25, 0.00562).$$

The drift τ^2 is the variance parameter that controls the amount of borrowing from one group to the next group. This parameter is estimated from the data and a prior distribution assuming a weight equivalent to 0.5 groups that the variance is equal to 0.15.

Lastly, a set of analyses will be fit with a spline on the log transformed baseline IL-6 values. Specifically, a penalized cubic order B-spline will be used with 10 knots and a random-walk prior that enforces smoothing on the spline coefficients. The prior on the basis coefficients $(a_1, \dots, a_B)$ will be specified as follows:

$$a_1 \sim N(0,1)$$
$$\alpha_i \sim N(a_{i-1}, \tau), i = 2, \dots, B$$
$$\tau \sim N^+(0,1)$$

Models will be fit with a spline estimating covariate effects of log baseline IL-6 and a spline of treatment effects for the pooled IL6Ra interventions by log baseline IL-6. The prior specifications for the treatment effects are the same as the covariate effects, using the same 10 knots and random walk prior.

10.4. Markov Chain Monte Carlo (MCMC) Model Stability

The Bayesian models have many parameters and there may be risk of poor model stability, including convergence and mixing behavior of the MCMC sampler. These instabilities may be based on sparse data on the outcome or covariates. The statisticians running the model may make changes that do not affect the overall interpretation but provide reliable model diagnostics and scientific rigor. Any alterations will be noted.

10.5. Model Outputs

The standard model outputs for each treatment effect will be the mean, standard deviation, median, and 95% credible intervals (all credible intervals will be equal-tailed intervals, so 95% credible intervals will range from the 2.5th percentile to the 97.5th percentile of the posterior distribution). For the ordinal/dichotomous endpoints, the odds-ratios will be summarized. For consistency, all models will be parameterized so that an odds-ratio greater than 1 indicates clinical benefit.

For each inferential model, a posterior probability that one arm is superior will be provided for each comparison between arms. This posterior probability has been identified as the primary analysis metric between arms.

11. Specific Prospective Analyses

The specific prospective analyses are summarized in the table and described in detail below.

#	Population	Endpoint	Covariates	Treatment Effects
OSFD1	Baseline IL-6 ITT	OSFD	Age, sex, site, time	Overall IL6Ra effect
OSFD2	Baseline IL-6 ITT	OSFD	Age, sex, site, time, baseline IL6 terciles	Overall IL6Ra effect
OSFD3	Baseline IL-6 ITT	OSFD	Age, sex, site, time, baseline IL6 terciles	Differential IL6Ra effect by baseline IL6 terciles
OSFD4	Baseline IL-6 ITT	OSFD	Age, sex, site, time, log baseline IL6 (NDLM)	Overall IL6Ra effect
OSFD5	Baseline IL-6 ITT	OSFD	Age, sex, site, time, baseline IL6 (NDLM)	Differential effect by baseline IL6 (NDLM)
OSFD6	Baseline IL-6 ITT	OSFD	Age, sex, site, time, log baseline IL6 (spline)	Overall IL6Ra effect
OSFD7	Baseline IL-6 ITT	OSFD	Age, sex, site, time, baseline IL6 (spline)	Differential effect by baseline IL6 (spline)
OSFD8	Baseline IL-6 ITT	OSFD	Age, sex, site, time, inflammatory subphenotype	Differential effect by inflammatory subphenotype
OSFD9	Baseline IL-6 ITT	OSFD	Age, sex, site, time, baseline IL6 terciles, baseline CRP terciles	Differential IL6Ra effect by baseline IL6 terciles
S1	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time	Overall IL6Ra effect
S2	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 terciles	Overall IL6Ra effect

S3	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 terciles	Differential IL6Ra effect by baseline IL6 terciles
S4	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 (NDLM)	Overall IL6Ra effect
S5	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 (NDLM)	Differential effect by baseline IL6 (NDLM)
S4	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 (spline)	Overall IL6Ra effect
S5	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, log baseline IL6 (spline)	Differential effect by log baseline IL6 (spline)
S6	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, inflammatory subphenotype	Differential effect by inflammatory subphenotype
S7	Baseline IL-6 ITT	Survival to hospital discharge	Age, sex, site, time, baseline IL6 terciles, baseline CRP terciles	Differential IL6Ra effect by baseline IL6 terciles

Inflammatory subphenotypes were identified based on unsupervised analyses of 26 biomarkers as described in Fish et al. (2022).

11.1. Reporting of Analysis Results

For each analysis model, the following summaries will be reported when applicable:

Parameter	Mean	SD	Median	95% Credible Interval
Age < 39				
Age 40-49				
Age 50-59				
Age 70-79				
Age 80+				
Female				
Time Bucket 1				
…				
Time Bucket k-1				
IL6Ra (versus control)				
Baseline IL-6				
Main effect of subgroup				
IL6Ra by subgroup				

11.2. Graphical summaries

The following graphical summaries will be provided for all endpoints:

- Population: Baseline IL-6 ITT
- Endpoint: all endpoints
- Factors: IL6Ra and Control; Baseline IL-6

12. REFERENCES

FISH, M., RYNNE, J., JENNINGS, A., LAM, C., LAMIKANRA, A. A., RATCLIFF, J., CELLONE-TREVELIN, S., TIMMS, E., JIRIHA, J., TOSI, I., PRAMANIK, R., SIMMONDS, P., SETH, S., WILLIAMS, J., GORDON, A. C., KNIGHT, J., SMITH, D. J., WHALLEY, J., HARRISON, D., ROWAN, K., HARVALA, H., KLENERMAN, P., ESTCOURT, L., MENON, D. K., ROBERTS, D., SHANKAR-HARI, M. & REMAP-CAP IMMUNOGLOBULIN DOMAIN UK INVESTIGATORS 2022. Coronavirus disease 2019 subphenotypes and differential treatment response to convalescent plasma in critically ill adults: secondary analyses of a randomized clinical trial. *Intensive Care Med,* 48**,** 1525-1538.