Statistical Analysis Plan

Study: VIRALBLOCK01

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Contents

Abbreviations

AE	Adverse Event
ADAM	Analysis Data Model
ANCOVA	Analysis of Covariance
CI	Confidence Interval
ITT	Intent-to-Treat
KM	Kaplan–Meier
LMM	Linear Mixed Model
PBO	Placebo
PSM	Propensity Score Matching
SAP	Statistical Analysis Plan
VRB	Viralblock

1 Introduction

This Statistical Analysis Plan (SAP) describes the planned analyses for the simulated randomized, double-blind, parallel-group clinical trial **VIRALBLOCK01**, evaluating the efficacy and safety of *Viralblock* compared with placebo in patients with a viral syndrome.

2 Objectives and Endpoints

2.1 Primary Objective

Assess the effect of Viralblock versus placebo on clinical recovery at Day 28.

2.2 Secondary Objectives

- Evaluate change in oxygen saturation (SpO_2) over 28 days.
- Assess time to hospitalisation or death.
- Compare incidence and severity of adverse events (AEs).
- Explore treatment effects across demographic and baseline covariates.

3 Study Design Summary

- Two-arm, 1:1 randomised, double-blind study.
- Sample size: 300 subjects (150 per arm).
- Populations: Intent-to-Treat (ITT), Safety, Per-Protocol (PP).

4 Analysis Populations

Safety: All randomised subjects who received at least one dose (`SAFFL='Y'`).

ITT: All randomised subjects (`ITTFL='Y'`).

PP: ITT subjects without major protocol deviations.

5 General Statistical Principles

Analyses will be performed using R (v4.4) and SAS (v9.4). Two-sided tests with $\alpha = 0.05$. Confidence intervals at 95%. Missing data handling in Section 7.

6 Statistical Methods

6.1 *t*-Test

The primary comparison of mean body temperature between treatment arms (Viralblock vs Placebo) at Day 7 will be conducted using a two-sample t-test.

The analysis will be based on the ADaM dataset `ADVS`, filtered for `PARAMCD="TEMP"` and `AVISIT="Day 7"`. The variable `AVAL` will be the analysis variable. The grouping variable is `TRTA` (Actual Treatment).

Assumptions of normality and equal variances will be visually assessed using:

- Boxplot of temperature distribution by treatment group
- Q–Q plots and histogram of residuals

The following outputs will be reported:

- Mean, standard deviation, and 95% confidence intervals per arm
- p-value for group comparison
- Graphical results saved in PDF (`ttest_temp_day7_full.pdf`)
- Numerical results exported in CSV (`ttest_temp_day7_results.csv`)

All results are stored in the project folder `4_analysis/analysis_outputs` and finalized figures in `5_results/final_tables_figures`.

6.2 ANCOVA

The primary objective of this analysis is to assess the adjusted mean change in oxygen saturation (SpO_2) from Day 1 (baseline) to Day 28 between treatment arms (Placebo vs Viralblock).
Endpoint: Change in SpO_2 (Day 28 – Day 1)
Population: Intent-to-Treat (ITT)
Model: A linear model was fitted with treatment arm (ARM), age, and sex as independent variables. ANCOVA was used to estimate least-squares means (LSMeans) for each treatment group and their adjusted difference.
Model specification:

$$\texttt{Change} = \beta_0 + \beta_1 \cdot \texttt{ARM} + \beta_2 \cdot \texttt{AGE} + \beta_3 \cdot \texttt{SEX} + \varepsilon$$

Output:

- Adjusted LSMeans for each group with 95% Confidence Intervals
- Treatment effect estimate (difference: VRB – PBO)
- Residual diagnostics, boxplots, and model fit plots

The outputs are available in the project repository under: `4_analysis/analysis_outputs/ancova/` and finalized in `5_results/final_tables_figures/ancova/`.

6.3 Logistic Regression

A binary logistic regression model was used to estimate the probability of clinical recovery (AEOUT = 'RECOVERED') by Day 28. The dependent variable was RECOVFL (Y/N). Independent variables included treatment group (ARM), age (AGE), and sex (SEX).

The model included:

- **Intercept**

- **Treatment (ViralBlock vs Placebo):** OR = 2.59 [95% CI: 1.44–4.67], p = 0.0015
- **Age:** OR = 1.005 [95% CI: 0.990–1.021], p = 0.5099
- **Sex (F vs M):** OR = 1.26 [95% CI: 0.70–2.27], p = 0.4347

Model diagnostics indicated a good fit (Likelihood Ratio Test p = 0.0101; AIC = 267.0). The treatment effect was statistically significant, suggesting that patients treated with ViralBlock had higher odds of recovery by Day 28 compared to placebo.

6.4 Time-to-Event Analysis

Endpoint. Time to first serious adverse event (SAE), defined as any adverse event with `AESER = "Y"`.

Population. All randomized subjects (`N = 300`) from the ADSL dataset.

Time Origin and Censoring. The time origin was defined as a proxy treatment start date set to `01-JAN-2022`. Subjects were censored at 28 days post-baseline unless a serious adverse event occurred earlier.

Kaplan–Meier Estimation. Kaplan–Meier curves were generated to compare survival (time to first SAE) between treatment arms (Placebo vs Viralblock). A log-rank test was used to evaluate statistical differences.
Result: No statistically significant difference was observed (log-rank p = 0.8130). See Figure 4 in Mock.

Cox Proportional Hazards Model. A Cox regression model was used to estimate the hazard ratio for the Viralblock group compared to placebo, adjusting for age and sex.
Model: `time*event(0) = ARM AGE SEX`
Hazard Ratio (HR): 0.855
95% CI: [0.395, 1.853]
p-value: 0.6912
Interpretation: No statistically significant effect of treatment was found. The confidence interval includes 1, indicating no clear evidence of reduced or increased hazard. See Figure 5 in Mock (Forest plot).

Repeated Measures Analysis of SPO2

To evaluate the evolution of oxygen saturation (SPO2) levels over time across treatment groups (Placebo vs. ViralBlock), two longitudinal analyses were conducted:

- **Repeated Measures ANOVA (RM-ANOVA)** was performed using data reshaped to wide format. SPO2 values collected at five visits (Days 1, 7, 14, 21, and 28) were modeled with treatment as a between-subject factor and time as a within-subject factor. Interaction effects were also assessed.

- **Linear Mixed Model (LMM)** was implemented on the long-format dataset using the SAS `PROC MIXED` procedure. The model included fixed effects for treatment group, visit number, and their interaction. A compound symmetry covariance structure was specified to model within-subject correlations. The estimation method used was REML.

Both models showed statistically significant effects for treatment group, time, and the interaction between treatment and time:

- RM-ANOVA (Time effect): $F(4, 1192) = 268.58$, $p < 0.0001$;
- RM-ANOVA (Time $\times$ Treatment interaction): $F(4, 1192) = 44.24$, $p < 0.0001$;
- LMM (Type III tests): all fixed effects $p < 0.0001$.

These findings suggest that SPO2 levels changed significantly over time and that the trajectory differed by treatment group.

Categorical Analysis

Adverse event (AE) presence and severity were analyzed using contingency tables stratified by treatment group. For binary outcomes (e.g., presence of any AE), comparisons between groups were conducted using the Chi-square test or Fisher's exact test when assumptions were violated. AE severity categories (e.g., mild, moderate, severe) were similarly compared across treatment arms using Chi-square tests.

Results. The presence of at least one AE did not differ significantly between treatment groups (Chi-square $p = 0.5281$; Fisher's exact $p = 0.5988$). Similarly, AE severity was not significantly associated with treatment (Chi-square $p = 0.3476$; Fisher's exact $p = 0.3584$).

A multinomial logistic regression was performed to assess the association between recovery status (RECOVERED, RECOVERING, NOT RECOVERED) and treatment group, adjusting for age and sex. None of the predictors showed statistically significant associations with recovery outcome at the 5% significance level. Odds ratios were estimated using a generalized logit link function, and the reference category was `RECOVERED`.

6. Propensity Score Matching

To address potential confounding in comparing the treatment groups (ViralBlock vs Placebo), a propensity score (PS) matching analysis was performed.

6.1 Propensity Score Estimation

A binary logistic regression model was used to estimate the propensity score, with treatment group (1 = ViralBlock, 0 = Placebo) as the outcome variable and `AGE` and `SEX` as predictors.

- Model: `treat` $\sim$ `AGE + SEX`
- Likelihood Ratio Test: $p = 0.5539$
- Estimated Odds Ratios:
  - AGE: OR = 1.002 (95% CI: 0.990–1.014), $p = 0.7444$
  - SEX (Male vs Female): OR = 1.273 (95% CI: 0.808–2.004), $p = 0.2976$
- Model discrimination: c-statistic = 0.534

6.2 Matching Procedure

Nearest-neighbor 1:1 matching without replacement was performed using the estimated propensity scores. A caliper width of 0.1 (on the logit scale) was applied to ensure similarity in matched pairs.

- Number of matched treated subjects: 137
- Number of matched control subjects: 137

6.3 Covariate Balance Diagnostics

Covariate balance before and after matching was assessed using standardized mean differences (SMD):

- SMD for AGE (after matching): 0.00 (100% reduction)
- SMD for SEX (after matching): 0.00 (100% reduction)
- SMD for logit(PS) (after matching): 0.0079

Several diagnostic plots were produced to evaluate balance:

- Distribution of propensity scores by treatment group
- Distribution of age by treatment group
- Bar plot of sex by treatment group
- Cloud plots of AGE and LPS

The diagnostics confirmed satisfactory balance between groups after matching.

8. Exploratory Multivariate Analysis: PCA and Clustering

An unsupervised exploratory analysis was performed to assess underlying patterns in the baseline characteristics of the study population, focusing on age and oxygen saturation (SPO2). Two statistical techniques were applied:

- **Principal Component Analysis (PCA)**: PCA was used to reduce dimensionality and to visualize potential correlations between AGE and mean SPO2 values. Subjects were projected onto the first two principal components, allowing identification of clusters or gradients in the population.
- **K-means Clustering (k=2)**: A k-means algorithm was applied to the standardized AGE and mean SPO2 values to partition the subjects into two clusters. The optimal number of clusters (k=2) was selected for interpretability and visualization. This clustering helps reveal latent groupings based on clinical profiles that may relate to treatment response or baseline severity.

Both analyses were conducted using R. Mean SPO2 values per subject were computed from the ADVS dataset (filtered by PARAMCD = "SPO2") and merged with demographic data from ADSL. Results were reported as biplots and cluster plots.

7 Missing Data

- Continuous: multiple imputation.
- Time-to-event: censoring at last contact.
- Sensitivity: tipping point analysis.

8 Multiplicity

No adjustment; secondary endpoints interpreted descriptively.

9 Software

R 4.4 and SAS 9.4. Scripts available in project GitHub repository.