# STATISTICAL ANALYSIS PLAN (SAP)

Feasibility study on the use of Redormin® 500 (Ze 91019) on day-time cognition and quality of life in people with occasional sleep problems

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| Study Type | Clinical Trial with Investigational Medicinal Product (IMP) |
| Study Categorization | Risk category B according to ClinO |
| Study ID | Ze 91019-04-2022-01 |
| Sponsor name | Zeller Medical AG<br>Seeblickstrasse 1<br>CH-8590 Romanshorn |
| CRO | GeneGuide AG<br>Birmannsgasse 8<br>CH-4055 Basel<br>Switzerland |
| Principal Investigator | Christiane Gerhards, MD<br>Research Platform MCN<br>Division Cognitive Neuroscience<br>University of Basel<br>Birmannsgasse 8<br>4055 Basel |
| Investigational Product | Redormin® 500 (Ze 91019) |

SAP and protocol version:

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| SAP version and date: | This SAP is version 2.0; 19. June 2024 |
| Protocol version | This document has been written based on information contained in the study protocol version 3.0, dated 15/02/2023 |

SAP revision history:

| Protocol version | SAP version | Section number changed | Description and reason for change | Date changed |
|---|---|---|---|---|
| 3.0 | 1.0 | | Description of exploratory analyses | 26 May 2024 |
| 3.0 | 2.0 | | Clarifications based on the feedback from Dr. J. Drewe | 19 June 2024 |
| 3.0 | 3.0 | 7 | Specification of the presentation of results (according to ICH E3 Guideline) | 26 June 2024 |

**Signature Page**

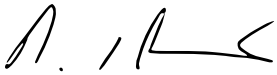
**Medical Director:**

Prof. Dr. Veronika Butterweck

Place/Date

**CRO:**

Prof. Dr. Dominique de Quervain



Place/Date Basel, 27.06.2024

Prof. Dr. Andreas Papassotiropoulos

Place/Date Basel, 27.06.2024

**TRIAL STATISTICIAN:**

Dr. Nathalie Schicktanz

Place/Date

Basel, 27. 06.2024

## Table of Contents

# 1 General Statistical Considerations

## 1.1 Statistical Framework

As defined in the protocol, the aim is to explore the feasibility of the study by using descriptive statistics of the variables listed under 9.2.1. of the study protocol. No a priori hypothesis is formulated. For secondary outcome variables, summary statistics will be provided (number of participants, frequency for categorical data, mean, SD, median, minimum and maxi- mum for continuous data). Additionally, we will conduct exploratory statistical analyses on the collected data.

Safety analysis: Adverse events will be listed and summarized.

## 1.2 Statistical Interim Analysis

There will be no interim analyses in this study.

## 1.3 Timing of Unblinding and Analyses

The primary analysis will be conducted once all subjects have completed the treatment period, the SecuTrial database is locked, and close out visit is done. Data from SecuTrial, SoSciSurvey, and Fitbit will then be transferred to LabKey. Within LabKey, quality control will be performed, and the data will be preprocessed and merged. The scripts for data processing and merging, as well as scripts to extract results for primary and secondary analyses will be stored and frozen before data unblinding, but not scripts for exploratory analyses.

# 2 Trial Population

## 2.1 Screening Data, Eligibility and Recruitment

A CONSORT flow diagram will be used to summarize the number of participants in the different phases of the study.

## 2.2 Baseline Patient Characteristics

The subjects' demographics and baseline characteristics to be summarized include age in years, gender, weight, height, BMI, sleep quality questionnaire (PSQI).

## 2.3 Withdrawal/Follow-up

This belongs to primary outcomes and will be listed by treatment group (see Consort Flow diagram).

## 2.4 Adherence and Protocol Deviations

### 2.4.1 Adherence to Allocated Treatment

Adherence to the use of the online toll SoSci-Survey, Fitbit and study medication will be assessed as part of primary outcomes on all 42 online diary visits.

### 2.4.2 Protocol Deviations

Protocol deviations are classified prior to unblinding of treatment. All protocol deviations are stored in LabKey.

### 2.5 Subjects Analyzed

Primary analyses will include data from all subjects interested in each type of advertisement up to the end of the study. Secondary and Exploratory analyses will include data from all randomized subjects without dropouts.

## 3 Outcome Definitions

### 3.1 Primary Outcome Definition

See section 9.2.1 of the study protocol.

### 3.2 Secondary Outcomes Definition

See section 9.2.2 of the study protocol.

## 4 Analysis Methods

### 4.1 Methods for the Primary Analysis of Primary and Secondary Outcomes

As defined in the study protocol, for the primary analysis, the number of subjects in the different study phases will be listed along with descriptive statistics of all other variables related to the feasibility of the study. For secondary outcomes, summary statistics will be provided (number of participants, frequency for categorial data, mean, SD, median, minimum and maximum for continuous data).

### 4.2 Methods for exploratory analyses

For the purpose of visual inspection, we will provide plots of the secondary outcome variables over all 21 visits per phase (run-in, treatment), displaying the mean ± SEM.

In the exploratory analyses, the treatment effect will be investigated using different statistical models. The software environment *R* will be used for statistical analyses (version 4.3.2, Rstudio).

**Model 1:**

- **Dependent Variables:** Means over the treatment phase of the secondary outcomes (labelled "mean_AV")
- **Covariates:** Age, sex, baseline (labelled "BL"; i.e. mean of a dependent variable over the last 7 visits in the run-in phase)
- **Independent Variable of Interest:** Treatment assignment (labelled "Assignment.treat"; i.e. assigned to which treatment; levels: Placebo or Verum)
- **Model:** mean_AV ~ sex + age + BL + Assignment.treat

We will use both linear (parametric) models and nonparametric models (Mann-Whitney U test). For nonparametric models, the dependent variable will be residualized for age, sex, and baseline (BL).

The assumption of normally distributed residuals will be tested using the *check_normality* function from the *performance* library, specifically with the Shapiro-Wilk test. A p-value less than 0.05 indicates a violation of the normality assumption.

**Model 2:**

- **Dependent Variables:** Secondary outcomes (labelled "AV")
- **Covariates:** Age, sex, baseline (labelled "BL"; i.e. mean of a dependent variable over the last 7 visits in the run-in phase)
- **Independent Variable of Interest:** Treatment assignment (labelled "Assignment.treat"; i.e. assigned to which treatment; levels: Placebo or Verum)
- **Other independent Variable:** visit number ("labelled sosci_link_nb", levels: 1 to 21)
- **Random effect:** Participant ID (labelled "1|ParticipantId") will be included as random effect
- **Model:** AV ~ sex + age + BL + sosci_link_nb + Assignment.treat + (1 | ParticipantId)

We will use both linear mixed-effects (*lme*) models (parametric) and similar nonparametric models (*nparLD* function from the *nparLD* package). Since *nparLD* does not allow missing values, missing values in dependent variables will be imputed by the median over the treatment phase within each subject. Prior to imputation the dependent variables will be residualized by age, sex, and baseline.

The normality of the residuals for both fixed and random effects will be tested using the *check_normality* function with the Shapiro-Wilk test. A p-value less than 0.05 indicates a violation of the normality assumption.

**Model 3:** Cognitive deficits are most likely to occur after the shortest nights. Therefore, we will investigate during the run-in phase if variables during/after the shortest night differ compared to all other nights.

- **Dependent Variables:** Secondary outcomes (labelled "AV")
- **Covariates:** age, sex.
- **Independent variable of interest:** visit (labelled "sosci_link_nb", levels: 22 = secondary outcome during/after the shortest night, and 23 = mean of the secondary outcome over the run-in phase without the shortest night).
- **Further independent variable:** treatment assignment (labelled "Assignment.treat"; levels: Placebo or Verum).
- **Random effect:** Participant ID (labelled "(1|ParticipantId)") will be included as random effect
- **Model:** AV ~ sex + age + sosci_link_nb + Assignment.treat + (1 | ParticipantId)

We will use both linear mixed-effects (lme) models (parametric) and nonparametric models (nparLD). In the case of non-parametric models (which do not allow missing values), the data frame will be reduced to include only subjects with values for the shortest night in the run-in phase. No imputation will be performed, as there is only one shortest night per participant. Given the unique nature of cognitive performance after the shortest night, any imputation may not reflect the true extent of cognitive deficits. Therefore, we prefer to exclude subjects with missing cognitive test data for the shortest night rather than risking inaccurate imputations. The dependent variables will be residualized for age and sex to enable the use of non-parametric nparLD models.

**Model 4:** Here, the treatment effect during/after the shortest night will be investigated, as cognitive deficits are most likely to occur after the shortest night.

- **Dependent variables:** Variables during/after the shortest night of the treatment phase (labelled "AV")
- **Covariates:** age, sex, baseline (labelled "BL", variable during/after the shortest night in the run-in phase)
- **Independent variable of interest:** treatment assignment (labelled "Assignment.treat"; levels: Placebo or Verum)

Model: AV ~ sex + age + BL + Assignment.treat

We will run both linear models (parametric) and nonparametric models (Mann-Whitney U test). For nonparametric models, the data frame will be reduced to subjects who have data after the shortest night in both the run-in and treatment phases. No imputation will be done, as there is only one shortest night. The dependent variable will be residualized for age, sex, and baseline (BL). Subsequently, the non-parametric Mann-Whitney U test will be performed to calculate effect sizes.

The assumption of normally distributed residuals of the models will be tested using the

*check_normality* function from the *performance* library, specifically employing the Shapiro-Wilk test on the residuals. A p-value of less than 0.05 indicates a violation of the normality assumption for the residuals.

Further statistical analyses may be proposed by the Sponsor or by the CRO.

## 5 Safety Analyses

Safety analyses will be based on the incidence and type of AEs. AEs will be tabulated and presented for all subjects and study phases, as well as summarized within the treatment phase only. The adverse events during the treatment phase will be investigated using Fisher's exact test to determine if there are significant differences between the placebo and verum groups.

## 6 Statistical Software

The software environment «R» will be used for statistical computing (version 4.3.2, Rstudio).

Following libraries will be used:

Data manipulation: *dplyr*, *conflicted, readxl*, *openxlsx, tidyr*, *plyr*, *dplyr*

Graphics: *ggpubr*, *ggplot2*

Following libraries will be used for exploratory analyses: *lme4*, *nlme*, *lmerTest*, *performance*, *r2glmm*, *emmeans*, *nparLD*, *conflicted*, *rstatix, lsr*

## 7 Presentation of the Results

The results, including tables with patient data listings, summary statistics, plots for visual inspection, and outcomes of the exploratory analyses, will be provided in a document formatted according to the ICH E3 Guideline.

## 8 References

Rstudio (2022). Share Everything R & Python Posit Connect. Available online at: http://www.rstudio.com/ (accessed October 12, 2022).