Statistical analysis plan

# COMB: A randomized, double -blind, placebo controlled, double -dummy, four -armed parallel -group, multicenter study during 12 -weeks to evaluate/comparing the effect of varenicline and bupropion in combination and alone for treatment of alcohol use disorder.

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# 1 INTRODUCTION

This statistical analysis plan (SAP) describes the (final) analyses to be performed at the account of Addiction Biology Unit. The SAP is based on the Clinical Study Protocol (CSP) Version 3.2, Lidö & deBejczy, 2020-06-16, with title "A randomized, double-blind, placebo-controlled multicenter trial on the efficacy of varenicline and bupropion, in combination and alone, for treatment of alcohol use disorder" and EudraCT No 2018-000048-24.

# 2 OBJECTIVES

## 2.1 Primary objective

To assess the effect and interaction of varenicline and bupropion in combination and alone versus placebo for reducing alcohol consumption in individuals with alcohol use disorder (AUD).

Two primary efficacy endpoints will be used

1) Alcohol consumption measured by the objective alcohol marker B-PEth
2) Alcohol consumption measured by proportion of heavy drinking days (HDD) by using the Time Line Follow Back (TLFB) procedure.

The primary objectives are analyzed in parallel.

## 2.2 Secondary objectives

The study includes the following secondary objectives:

1) To assess the effect and interaction of varenicline and bupropion in combination and alone versus placebo for reducing alcohol consumption in individuals with AUD by using additional biological markers
   - carbohydrate deficient transferrin (CDT)
   - gamma glutamyltransferase (GGT)

2) To assess the effect and interaction of varenicline and bupropion in combination and alone versus placebo for reducing alcohol consumption in individuals with AUD by using self-reported alcohol consumption measured by TimeLine Follow Back (TLFB):
   - Mean grams alcohol per day
   - Number of drinking days
   - Number of abstinent days

- Number of drinks per drinking day

3 To assess the effects of varenicline and bupropion in combination and alone versus placebo, on other clinically relevant efficacy endpoints:
  - Total score of Alcohol Use Disorder Identification Test (AUDIT), and sub score
    AUDIT -C
  - Alcohol craving measured by Visual analogue scale (VAS)
  - Nicotine use measured by subjective nicotine use from questionnaire
  - Temporal experience of pleasure scale (TEPS)

- To assess the relationships between the above described outcome measures and plasma drug concentrations of BUP and VAR measured at visit 4 and 6

# 3 STUDY DETAILS

## 3.1 Study design

Study design is described in detail in the CSP (version 3.2, 2018-02-09).

The study is designed as a Phase IIB, randomized, double-blind, placebo-controlled multicenter trial with 4 parallel groups, evaluating the effects of varenicline (drug A) and bupropion (drug B) in combination and alone compared to placebo for treatment of alcohol use disorder (AUD). (Arm 1: drug A+drug B, Arm 2: drug A+placebo, Arm 3: drug B+placebo, Arm 4: placebo + placebo). The treatment effect will be measured by change in alcohol consumption and the difference between treatment groups.

Duration: 13 weeks

## 3.2 Sample size

The power analysis is based on two independent primary hypotheses;

- PEth differ significantly between varenicline and bupropion in combination and alone versus placebo
- Proportion of HDD differ significantly between varenicline and bupropion in combination and alone versus placebo

With regards to PEth, we assume that we can use an ordinary t-tests based on normal distribution, with $\alpha$=0.05 (two-sided), 1-$\beta$=0.80 and using a standard deviation of 0.8 Recruiting 320 patients, randomized in 4 groups of 80 each, we get a detectable effect of 0.35 umol/l and a Cohen's d of 0.44.

With regards to HDD, we assume that we can use ordinary t-tests based on normal distribution, with α=0.05 (two-sided), 1-β=0.80, using a standard deviation of maximum 0.35. Recruiting 360 patients, randomized in 4 groups of 90 each, we get a detectable effect of 15% and a Cohen's d of 0.43.

Hence, a sample size of 360 subjects should be randomized.

## 3.3 Drop-out rate

Based on prior studies (among other, Varenicline for treatment of alcohol dependence, A de Bejczy, ACER 39(11):2189-2199, 2014) with similar design and outcome, the drop-out rate is estimated to approx. 20 % between screening and randomization and approx. 6.5% from randomization to subjects included in ITT. To balance the drop-out 380 subjects are estimated for screening and 360 subjects for randomization.

## 3.4 Centers

The data is collected from 4 centers.

# 4 ANALYSIS SETS

## 4.1 Definition of analysis sets

Three analysis sets will be used: modified Intention-To-Treat m(ITT), Per Protocol (PP) and Safety. The primary and secondary efficacy endpoints will be estimated using mITT and PP analysis sets separately. The safety endpoints will be analyzed on the safety analysis set.

### 4.1.1 Modified Intention -to-treat (mITT) analysis set

The modified Intention To Treat (mITT) analysis set constitutes all trial subjects who complete screening visit, and randomization visit and report that they have taken at least one dose of randomized investigational medicinal product (IMP). Subjects will be analyzed according to their randomized IMP.

### 4.1.2 Per-protocol (PP) analysis set

The PP analysis set constitutes all subjects from the mITT population who comply to the study regimen, *i.e.*

has taken the IMP at least 80% of the planned number of doses for the treatment period starting 1 day after visit 2 (randomization) to visit 8. A detailed description of the pill count criteria is given in Appendix D.

Also, for the primary endpoint PEth the subject needs to have successful PEth analyses at all of the following milestones:

- Screening,
- Visit 4,
- Visit 5 or visit 6 (either or both) and
- Visit 7 or visit 8 (either or both)

Also, for the primary endpoint HDD the subject needs to have successful TLFB procedure at all of the following milestones:

- Screening,
- Visit 4,
- Visit 5 or visit 6 (either or both) and
- Visit 7 or visit 8 (either or both)

In case of violation of any of the above-mentioned criteria the subject will not be included in the respective PP analysis set. Subjects will be analyzed according to their randomized IMP.

Due to the special circumstances during the Covid-19 pandemic 2020-21 the authorities have accepted special adjustment to clinical trials (see *eg* EMA: GUIDANCE ON THE MANAGEMENT OF CLINICAL TRIALS DURING THE COVID-19 (CORONAVIRUS) PANDEMIC). Some of the participating sites were inhibited to collect PEth values from test subjects due to visiting restrictions, meanwhile HDD could be collected over phone. Therefore, the participants unable to deliver PEth-measurements due to covid19 lockdown could still be included in PP analyses set with regards to HDD analysis. We will create two per propocol populations according to the following:

- Per protocol set 1 (PEth): Subjects in mITT data set fulfilling milestones for PEth PP analysis set, regardless of failure to fulfill milestones for HDD PP analysis set.
- Per protocol set 2 (HDD): Subjects in mITT data set fulfilling milestones for HDD PP analysis set, regardless of failure to fulfill milestones for PEth PP analysis set.

The inclusion in the per protocol data sets is decided at the clean file meeting.

### 4.1.3 Safety set

The safety analysis set constitutes all subjects who report that they have taken at least one dose of randomized study drug, and for whom any post-dose data are available up until Visit 9.

Subjects will be analyzed according to their randomized IMP.

## 4.2 Violations and deviations

Violations and deviations will be described in the CSP (version 3.2, Lidö & de Bejczy, 2018-02-09).

### 4.2.1 Compliance

Compliance to the study drug regimen will be analyzed by pill count and by blood levels of drug/placebo markers.

### 4.2.2 Randomization issues

Mistakenly randomizing a subject who does not meet the inclusion/exclusion criteria will be considered a protocol violation. Any such patients will not be included in the analysis sets.

## 4.3 Clean file meeting

A clean file meeting will be held before data is unblinded, agenda is described in the DMP. The meeting will be called by the principal investigator, data management is responsible for taking minutes from the meeting and providing a clean file report.

Data is unblinded after declared clean file and data lock.

# 5 PRIMARY AND SECONDARY ENDPOINTS

## 5.1 Primary endpoints

The study is using two primary endpoints (PEth levels and proportion of HDD). They are expected to be correlated, but due to different requirements both are needed for the analysis.

### 5.1.1 PEth

One of two primary endpoints will be the levels of the objective alcohol marker Phosphatidylethanol (PEth) measured in blood [µmol/l].

PEth will be collected initially at the screening visit (visit 1), at randomization visit (visit 2), after one week of titration (visit 3) and for steady state period every second week (visit 4-visit 9).

The primary efficacy endpoint, PEth, is calculated as the absolute difference in mean value over the 8-week steady state active treatment period visit 4-visit 8 compared to baseline (visit 1). The data is analyzed as the within subject difference.

PEth has a well-documented [see CSP, Version 3.2, 2018-02-09) declining pattern and hence it follows that the mean value during the treatment period will be a conservative statistic for the post-treatment level.

### 5.1.2 Heavy Drinking Days (HDD)

One of two primary endpoints will be alcohol consumption as measured by proportion of Heavy Drinking Days (HDD) using the Time Line Follow Back (TLFB) procedure.

TLFB will be collected initially at the screening visit (visit 1), at randomization visit (visit 2), after one week of titration (visit 3) and for steady state period every second week (visit 4-visit 9).

The baseline proportion of HDD is obtained at visit 1, where the number of heavy drinking days over the last 30 days is recorded using TLFB. However, only the 14 days directly prior to the screening visit are used for estimation of baseline HDD.

A heavy drinking day is defined as a day, where a male subject has consumed ≥ 70g alcohol (5 units à 14 g) or a female subject has consumed ≥ 56g alcohol (4 units à 14 g). The alcohol consumption in gram is based on the conversion standard from TLFB.

The proportion of HDD in the TLFB is defined as

$$Prop(HDD) = \frac{Number\ of\ HDD}{Number\ of\ days\ in\ TLFB}.$$

HDD is evaluated by comparing the absolute difference in proportion of HDD between visit 4-visit 8, compared to baseline (visit 1).

## 5.2 Secondary efficacy endpoints

Secondary endpoints will include:

- Additional alcohol markers defined as carbohydrate deficient transferrin (CDT) and Gamma glutamyltransferase (GGT) measured in blood
- Self-reported alcohol intake measured by TLFB and analyzed by:
  - Mean grams alcohol per day
  - Number of drinking days
  - Number of drinks (units à 14 g) per drinking days
  - Number of abstinent days
- Alcohol Use Identification test (AUDIT)
  - AUDIT-C, using the score of the first 4 items of the AUDIT questionnaire
  - AUDIT-Total, using the score of the full AUDIT questionnaire
- Alcohol Craving measured by Visual analogue scale (VAS)

- Nicotine use according to subjective assessment:
  - Daily nicotine (yes/no)
  - Total nicotine use per day
- Temporal experience of pleasure scale (TEPS)
- To assess the relationships between the above described outcome measures and plasma drug concentrations of BUP and VAR measured at visit 4 and 6

Primary endpoints (PEth and HDD) will be calculated and presented descriptively at all visits.

# 6 STATISTICAL AND ANALYTICAL PLANS

## 6.1 General principles

All efficacy and safety endpoints will be presented using descriptive statistics and graphs as appropriate. Safety measures will be summarized descriptively using the safety analysis set. Continuous variables will be presented with descriptive statistics (n, mean, standard deviation [SD], median, min, max), within treatment group. Categorical variables will be summarized by treatment group in frequency tables (number of patients and percentage). The denominators for all percentages will be defined as the number of non-missing values for each variable within the analysis set. Percentages will be presented with 1 decimal place.

The R software, version 4.2.2, will be used to produce analyses, figures and tables.

### 6.1.1 Baseline

Baseline values will be collected at the screening visit (Visit 1).

### 6.1.2 Treatment period

The steady-state active treatment period is defined as visit 4 until visit 8 and primary efficacy analysis and secondary endpoint measures will be analyzed for this period.

In the steady state active treatment period data from visit 2 (randomization visit) is not included as IMP start is the day after visit 2. Between visit 2 and 3 the IMP titration occurs and therefore visit 3 is excluded. At visit 4 steady state is achieved. Data from visit 9 is excluded due to a higher expected drop-out rate and non-compliance IMP.

### 6.1.3 Follow -up Period

The follow-up period constitutes the period from the day after the treatment period ends (EoT visit 9) until the last follow-up phone call (FU30). The follow-up period is targeted to last for 30 days.

### 6.1.4 Multiplicity

No adjustment for multiplicity will be done for the two primary endpoints. The overall type 1 error rate will be 10% (two-sided).

Testing between treatments for each of the primary endpoints are performed separately, and for each in a hierarchical fashion,

### 6.1.5 Handling of missing data

In a blind data review, missing data will be assessed, see section 8.2.

It is expected that PEth and HDD, for successful treatment, will decline over time. Subjects, not following the full treatment period, will therefore be analyzed as long as there is data available. This is expected to give an underestimation of the treatment effect.

Secondary analyses will be made on the available data, *ie* mean values are calculated on the data that is present.

No imputation will be done.

### 6.1.6 Patient disposition

A summary table and CONSORT diagram, by treatment arm and overall, will be presented for the number of patients who have been:

- Enrolled
- Randomized
- Received at least one dose of randomized IP
- Did not receive treatment
- Completed the study
- Prematurely discontinued the study (along with the categorized reason for discontinuation)

The number of patients included in each of the analysis sets will also be tabulated, as well as reasons for exclusion from each analysis set.

### 6.1.7 Protocol Deviations

A list of important protocol deviations will be finalized prior to study un-blinding. The number and percentages of subjects with at least one important protocol deviation will be summarized by treatment arm and overall. The number and percentages of subjects within each type of protocol deviation will be provided as well.

A listing of all subjects with any important protocol violations/deviations will be provided for them mITT population.

## 6.2 Blind data review

A blind data review will be performed while data is still double blind according to the DMP.

The number of missing data and data quality will be assessed.

Number of valid data for PEth and HDD during treatment period is screened. Number of subjects excluded (see Section 6.1.6) is reported. If the drop-out rate is considered high, analyses criteria will be reviewed.

## 6.3 Analysis of primary endpoints

Primary endpoints will be analyzed according to mITT and will be summarized descriptively. Analysis will be presented in tables presenting estimates of the coefficients, their 95 % confidence intervals and p-values. All tests will be two-sided, 5% significance level. When reporting the results of significance tests, precise p-values will be reported.

Primary endpoints (PEth and HDD, respectively) will also be analyzed with the same approach described below using the PP analysis set.

Other post-hoc tests will be used if needed and as appropriate.

### 6.3.1 Hierarchal testing procedure

The analysis for PEth and HDD compares the mean values of the variable in question for the different treatment groups hierarchically:

Step1: $H_{01}$: A+B = $\text{placebo}_A$+$\text{placebo}_B$

$H_{11}$: A+B ≠ $\text{placebo}_A$+$\text{placebo}_B$

If significant difference (2 sided, 5% significance level) proceed to step 2.

Step2: $H_{02}$: A+ $\text{placebo}_B$ = $\text{placebo}_A$+$\text{placebo}_B$

$H_{12}$: A+ $\text{placebo}_B$ ≠ $\text{placebo}_A$+$\text{placebo}_B$

If significant difference (2 sided, 5% significance level) proceed to step 3.

Step3: $H_{03}$: A+B = A+$\text{placebo}_B$

$H_{13}$: A+B ≠ A+$\text{placebo}_B$

If significant difference (2 sided, 5% significance level) proceed to step 4.

Step4: $H_{04}$: A+B = B+$\text{placebo}_A$

$H_{14}$: A+B ≠ B+$\text{placebo}_A$

If significant difference (2 sided, 5% significance level) proceed to step 5.

Step5: $H_{05}$: B + $\text{placebo}_A$ = $\text{placebo}_A$ +$\text{placebo}_B$

$H_{15}$: B + $placebo_A \neq placebo_A + placebo_B$

### 6.3.2 PEth

An analysis with regards to PEth, will be performed using a regression model with the absolute difference in mean value of PEth, within subject, between visit 4 – 8 and visit 1

$$diff(PEth) = mean(Visit\ 4\ to\ 8) - (Visit\ 1\ ) =$$
$$= \frac{Peth(4) + Peth(5) + Peth(6) + Peth(7) + Peth(8)}{count\ valid\ PEth\ during\ visit\ 4\ to\ 8} - Peth(1)$$

The treatment effect will be estimated, with adjustments for Gender and baseline PEth.

$$diff\ (PEth) = \beta_0 + \beta_1 \cdot Treatment + \beta_2 \cdot Gender + \beta_3 \cdot Baseline(PEth)$$

$\beta_0$ is a constant,

$\beta 1$ is the coefficient for treatment group,

$\beta_2$ is the coefficient for gender (Male/Female),

$\beta_3$ is the linear coefficient for baseline PEth

Standard analyses of the residuals will be performed to evaluate that the assumptions for regression models are fulfilled. If assumptions of normality are not met, a non-parametric analysis will be made to study the robustness of the regression analysis.

### 6.3.3 Heavy Drinking Days (HDD)

An analysis with regards to proportion of HDD, will be performed using a regression model with the absolute difference in mean value of proportion of HDD, within subject, between visit 4 -8 and visit 1

$$diff(propHDD) = mean\ (Visit\ 4\ to\ 8) - visit\ 1 =$$
$$= \frac{propHDD(4) + propHDD(5) + propHDD(6) + propHDD(7) + propHDD(8)}{count\ valid\ propHDD\ during\ visit\ 4\ to\ 8} - propHDD(1)$$

HDD is calculated from TLFB 14 days prior to visit

The treatment effect will be estimated, with adjustments for Gender and baseline HDD.

$$diff\ (propHDD) = \beta_0 + \beta_1 \cdot Treatment + \beta_2 \cdot Gender + \beta_3 \cdot Baseline(HDD)$$

$B_0$ is a constant,

$\beta 1$ is the the coefficient for treatment group,

$\beta_2$ is the coefficient for gender (Male/Female),

$\beta_3$ is the linear coefficient for baseline HDD

Standard analyses of the residuals will be performed to evaluate that the assumptions for regression models are fulfilled. If assumptions of normality are not met, a non-parametric analysis will be made to study the robustness of the regression analysis.

## 6.4 Analysis of secondary endpoints

Secondary endpoints will be analyzed according to mITT and PP.

### 6.4.1 Development of PEth and HDD during treatment period

Both mean PEth-level and proportion of HDD will be presented graphically for visit 1 to visit 9, split by treatment group.

All secondary endpoints will be presented graphically for visit 1 to visit 9, split by treatment group.

### 6.4.2 Additional alcohol markers

Additional alcohol markers CDT [%] and GGT [µkat/l] will be collected at every visit.

They will be analyzed as the absolute difference in mean value over the 8-week steady state active treatment period visit 4-visit 8 compared to visit 1 (Baseline). The data is analyzed as the within subject difference.

Data will be analyzed descriptively and statistical comparisons between treatments will be performed by a t-test or Wilcoxon test, depending of distribution and sample size.

### 6.4.3 Subjective alcohol consumption (TLFB)

Further results from Time Line Follow Back (TLFB) of subjective alcohol consumption will be estimated from TLFB:

- Mean grams alcohol per day [g/day],
- Number of drinking days, *ie* days with the alcohol consumption > 0
- Number of abstinent days, *ie* days with the alcohol consumption = 0
- Number of drinks per drinking day, *ie* mean standard units à 14 g alcohol, for days with the alcohol consumption > 0

They will be analyzed as the absolute difference in mean value over the 8-week steady state active treatment period visit 4-visit 8 compared to visit 1 (Baseline). The data is analyzed as the within subject difference.

Baseline of TLFB will be derived using TLFB14 at screening.

Data will be analyzed descriptively and statistical comparisons between treatments will be performed by a t-test or Wilcoxon test, depending of distribution and sample size.

### 6.4.4 AUDIT

AUDIT will be measured at visit 1 and at visit 8 using standard questionnaire. In the analysis both AUDIT-C (first 4 questions) and AUDIT (total) will be analysed.

Change from baseline will be calculated and analyzed descriptively and statistical comparisons between treatments using a Wilcoxon test.

### 6.4.5 Alcohol craving (VAS)

Alcohol craving will be measured by Visual analogue scale (VAS).

Data will be analyzed as the absolute difference in mean value over the 8-week steady state active treatment period visit 4-visit 8 compared to visit 1 (Baseline). The data is analyzed as the within subject difference.

Data will be analyzed as a mean over the active treatment period (visit 4 - visit 8). Change from baseline will be calculated. Data will be analyzed descriptively and statistical comparisons between treatments will be performed by a t-test or Wilcoxon test, depending on distribution and sample size.

### 6.4.6 Daily nicotine use

Nicotine use is measured subjectively at visit 1 and visit 8. The following endpoints are defined:

- Daily Nicotine (Yes/No) and
- Total number of nicotine (any form) per day, change from baseline will be calculated.

Data will be analyzed descriptively and statistical comparisons between treatments will be performed.

The first endpoint (yes/no) will be analyzed using a $\chi 2$ test.

The difference between visit 1 and 8 for the second endpoint (total number of nicotine usings) will be analyzed by a t-test or Wilcoxon test, depending of distribution and sample size.

### 6.4.7 Temporal experience of pleasure scale (TEPS)

Temporal experience of pleasure scale (TEPS) will be measured at visit 1 and visit 8, change from baseline will be calculated.

Data will be analyzed descriptively and statistical comparisons between treatments will be performed by a t-test or Wilcoxon test, depending of distribution and sample size.

## 6.5 Other analyses

### 6.5.1 Study medication

Concentration of study medication in plasma blood will be evaluated at all visits: Bupropion [ng/ml] and Varenicline [ng/ml], respectively.

### 6.5.2 Interaction of alcohol and nicotine consumption

Interaction between alcohol and nicotine consumption will be analyzed.

### 6.5.3 Interaction between primary and secondary endpoints

Exploratory analyses, correlation analysis, will be performed to assess the relationship between the primary and secondary endpoints, respectively.

### 6.5.4 Interaction between endpoints and study drug

Exploratory analyses, correlation analysis, will be performed to assess the effects of the primary and secondary endpoints in relation to blood drug concentrations of BUP and VAR.

### 6.5.5 Development of primary endpoints over time

A graphical presentation of the primary endpoints (PEth and HDD) will be made over time (visits) and treatment groups. Further statistical analyses to compare treatments will be made.

### 6.5.6 Multiple regression of primary outcomes vs demographics

Exploratory analyses, correlation analysis, will be performed to assess the treatment effects of the primary endpoints in relation to demographic data.

## 6.6 Subgroup for exploratory analysis

Exploratory analyses will be performed on the impact of demographic data on the above described endpoints. Treatment effects will be analysed in relation to

- Gender,
- Family History Positive (FHP)
- Consumption levels, defined by the following limits for male subjects:
  - *Low risk: 0-40 g/day,*
  - *Moderate risk: 41-60 g/day,*
  - *High risk: 61-100 g/day and*
  - *Very high risk: at least 101 g/day*
- Consumption levels, defined by the following limits for female subjects:
  - *Low risk: 0-20 g/day,*

  - *Moderate risk: 21-40 g/day,*
  - *High risk: 41-60 g/day and*
  - *Very high risk: at least 61 g/day*
- Early responders, defined by a 50% or more reduction in alcohol between visit 1 (screening) and visit 2 (randomization/start IMP).

## 6.7 Sensitivity analysis

Sensitivity analyses of primary endpoints will be performed with regards to both PEth and HDD. A complete case analysis will be performed using all subjects with a full set of study data with regards to PEth and HDD.

A sensitivity analysis will be conducted to evaluate whether a between center effect is present.

The sensitivity analysis is done the following way:

Primary endpoints, mean difference in PEth and HDD, is analyzed using two way ANOVA with Center and Treatment as independent factors. ANOVA is presented with p-values for both independent factors for descriptive evaluation purposes.

If the Center factor is not significant, *ie* has $p < 0.05$, the primary endpoints are also evaluated using a regression model used in the primary analysis: The treatment effect will be estimated, with adjustments for Gender and baseline PEth and HDD, respectively.

$$diff\ (PEth) = \beta_0 + \beta_1 \cdot Treatment + \beta_2 \cdot Gender + \beta_3 \cdot Baseline(PEth)$$

$\beta_0$ is a constant,

$\beta_1$ is the coefficient for treatment group,

$\beta_2$ is the coefficient for gender (Male/Female),

$\beta_3$ is the linear coefficient for baseline PEth

$$diff\ (propHDD) = \beta_0 + \beta_1 \cdot Treatment + \beta_2 \cdot Gender + \beta_3 \cdot Baseline(HDD)$$

$B_0$ is a constant,

$\beta_1$ is the the coefficient for treatment group,

$\beta_2$ is the coefficient for gender (Male/Female),

$\beta_3$ is the linear coefficient for baseline HDD

Results are presented as an ANOVA, where p-values are presented for descriptive evaluation purposes.

# 7 INTERIM ANALYSIS

NA

# 8 CHANGES OF ANALYSIS FROM EARLIER VERSIONS OF PROTOCOL AND SAP

## 8.1 Models for primary endpoints

In earlier versions of the SAP a multivariate mixed model would have been applied for the primary analysis. It consisted of Gender, Family History Positive (FHP), Nicotine use and Baseline value of HDD and PEth, respectively.

After consulting FDA guidelines[1] we have come to the conclusion that the models in the primary analysis should be kept as simple as possible. This is to promote such a conservative and low powered estimation of treatment effect as possible.

We conclude that:

- Gender is known to have possible effects on treatment for addiction disorders and can therefore be kept in the model. It is important to the study, to know whether gender will cause specific treatment recommendations. And since HDD cutoffs are gender specific, there is a possible correlation between gender and baseline HDD that needs to be taken into account.
- It is recommended from FDA that baseline values are taken into the model. It is motivated from the fact that the treatment effect, in absolute numbers, can be depending on the baseline value.
- Nicotine is included in the secondary analyses and hance cannot be used as a covariate in the primary analysis.
- FHP is omitted from the model due to the possible difficulties to interpret and apply the results if it were significant.

Further, we believe it would not be appropriate to model gender, FHP or nicotine use as random effects if they were included, as

(i) our sampling of levels from these variables is exhaustive

(ii) the variables are dichotomous

The variables have sufficient sample size for each level. Thus, all effects should be modelled as fixed.

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1) *FDA (2021). Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products; Guidance for Industry.*

The primary analyses will be done using a multiple regression model with Gender and Baseline value PEth and HDD, respectively.

## 8.2 Removal of CPTA as secondary endpoint

CPTA is a innovative and novel way of detecting ADHD among test subjects. It is based on a hand held app to be installed on a standard smartphone. The software is developed by Opatus AB (www.opatus.se).

The CPTA test score was not validated clinically versus verified golden standards, when the test subjects were recruited but was expected to have a finalized validation by the time of data base lock. Since CPTA score is not scientifically validated it has been removed as secondary endpoint.

Since no other model or endpoint is estimated using CPTA score we conclude that the removal will not affect the general validity and relevance for the study.

Section 6.5.2. is removed. Sections 6.5.3 – 7 is renumbered.

## 8.3 Analysis of center (site) effect

Section 6.7 specifies that an analysis of the difference between centers or sites to the primary endpoints. The detailed analysis is amended to the Section.

# 9 REPORTING AND PUBLICATION OF RESULTS

According to agreement with Addiction Biology Unit

# Appendix A. Tables, Figures and Listings

### Table 1. Listing of Tables

| Table Number | Table Title |
|---|---|
| 1 | Baseline characteristics – quantitative data |
| 2 | Baseline characteristics – frequency data |
| 3 | Count of AE, SAE and subjects with AE/SAE over time |
| 4 | Count of AE, SAE by organ class |
| 5 | Count of medical history according to MedDRA |

| Table Number | Table Title |
|---|---|
| 6 | Count of concomitant medication according to ATC |
| 7 | Primary endpoint PEth, difference baseline vs Visit 4-8, sequential tests (Section 6.3.1) |
| 8 | Primary endpoint HDD, difference baseline vs Visit 4-8, sequential tests (Section 6.3.1) |
| 9 | CDT, absolute difference between baseline vs Visit 4-8 |
| 10 | GGT, absolute difference between baseline vs Visit 4-8 |
| 11 | Subjective alcohol consumption (absolute difference between baseline vs Visit 4-8):<br>• Mean grams alcohol per day,<br>• Number of drinking days,<br>• Number of abstinent days,<br>• Number of drinks per drinking day |
| 12 | AUDIT-C and AUDIT-total at baseline, visit 8 and within subject difference |
| 13 | Alcohol craving at baseline, visit 8 and within subject difference |
| 14 | Nicotine use at baseline, visit 8 and within subject difference<br>• Daily nicotine use (yes/no),<br>• Total number of nicotine (any form) per day |
| 15 | TEPS at baseline, visit 8 and within subject difference |
| 16 | Concentration of study medication in plasma blood<br>• Bupropion<br>• Varenicline |

Table 2. Listing of Figures

| Figure Number | Figure Title |
|---|---|
| 1 | CONSORT diagram |
| 2 | PEth concentration over time (visit 1-9), group by treatment |

| | |
|---|---|
| 3 | HDD proportion over time (visit 1-9), group by treatment |

Table 3. Listing of Listings

| List Number | List Title |
|---|---|
| 1 | Reported protocol deviations |
| 2 | Reported SAE and AE events |

# Appendix B. List of abbreviations

| Abbreviation | |
|---|---|
| AE | Adverse event |
| ADHD | Attention Deficit Hyperactivity Disorder |
| ANOVA | Analysis of Variance |
| AUDIT | Alcohol Use Disorder Identification Test |
| CDT | Carbohydrate Deficient Transferrin |
| CPTA | Continous Performance Test – Activity |
| CSP | Clinical Study Protocol |
| DMP | Data Management Protocol |
| DUDIT | Drug Use Disorder Identification Test |
| EoT | End of Treatment |
| FHP | Family History Positive |
| FU | Follow up |
| GGT | Gamma Glytamyltransferase |
| HDD | Heavy Drinking Days |
| IMP | Investigational Medicinal Product |
| ITT | Intention to Treat |
| LOCF | Last Observation Carried Forward |
| MAR | Missing At Random |
| MNAR | Missing Not At Random |
| NA | Not Applicable |
| PEth | Phosphatidylethanol |
| PP | Per Protocol |
| SAE | Severe adverse event |
| SAP | Statistical Analytical Plan |
| SD | Standard Deviation |
| TEAE | Treatment emergent adverse event |
| TLFB | Time Line Follow Back |
| U-EtG | Urine Ethyl Glucoronide |
| VAS | Vislual Analogue Scale |

# Appendix C. **Schedule of assessments**

Table 4. Time schedule and summary of study assessments

| Accepted range | | <+14 days | <+8 days | +/- 3 days | +/- 3 days | +/- 3 days | +/- 3 days | +/- 3 days | +/- 3 days | +/- 3 days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of study visits: 9 | Ph Scr | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 | Visit 9 | Ph call | Ph call |
| Total on-site study visit time: 13 hrs. | | 2.5 hrs | 2 hrs | 1 hr | 1 hr | 1 hr | 1.5 hrs | 1 hr | 1.5 hr | 1.5 hrs | 15m | 15m |
| Total physician time 2.5 hrs. | | 1 hr | 30m | | | | 30m | | | 30m | | |
| Eligibility assessments | | Scr | Day 0 | Day 7 | Day 21 | Day 35 | Day 49 | Day 63 | Day 77 | Day 91 | FU +7 | FU +30 |
| Informed consent | | √ | | | | | | | | | | |
| ID Control | | √ | | | | | | | | | | |
| Breath alcohol concentration | | √ | | | | | | | | | | |
| Subject eligibility criteria | √ | √ | √ | | | | | | | | | |
| Randomization | | | √ | | | | | | | | | |
| Patient trial card distribution | | | √ | | | | | | | | | |
| Patient trial card collection | | | | | | | | | | √ | | |
| Demographics | | √ | | | | | | | | | | |
| Medical history | √ | √ | | | | | | | | | | |
| Concomitant medication | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| Physical examination * | | √ | √ | | | | √* | | | √ | | |
| Vital signs | | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| ASRS | | √ | | | | | | | | | | |
| WURS | | √ | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MADRS (also safety measure) | | √ | | | √ | | | | | √ | | |
| HAM-A | | √ | | | | | | | | | | |
| DUDIT (also safety measure) | | √ | | | | | | | | √ | | |

| Outcome specific measurements | | Scr Visit 1 | Day 0 Visit 2 | Day 7 Visit 3 | Day 21 Visit 4 | Day 35 Visit 5 | Day 49 Visit 5 | Day 63 Visit 7 | Day 77 Visit 8 | Day 91 Visit 9 | FU+7 | FU+30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TLFB alcohol | | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| VAS Craving | | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| B-Peth | | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| CDT | | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| GGT | | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| Alcohol QF | √ | √ | √ | | | | | | | | | |
| Nicotine QF | √ | √ | √ | | | | | | | | | |
| Alcohol history | | √ | | | | | | | | | | |
| Nicotine history | | √ | | | | | | | | | | |
| AUDIT | | √ | | | | | | | √ | | | |
| Cotinine | | | √ | | | | | | √ | | | |
| CPTA test | | | √ | | | | | | √ | | | |
| Breath alcohol concentration | | √ | √ | | | | | | √ | | | |
| TEPS scale | | | √ | | | | | | √ | | | |
| Drug plasma concentration BUP | | | | | √ | | √ | | | | | |
| Drug plasma concentration VAR | | | | | √ | | √ | | | | | |

| Safety measurments | | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 | Visit 9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AE/SAE | | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| AST | | √ | | | | | | | | √ | | |
| ALT | | √ | | | | | | | | √ | | |
| Glucose | | √ | | | | | | | | √ | | |
| Creatinine | | √ | | | √ | | | | | √ | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PC (prothrombin complex) | | √ | | | √ | | | | | √ | | |
| Na+/K+ | | √ | | | | | | | | √ | | |
| Hb | | √ | | | | | | | | √ | | |
| LPK | | √ | | | | | | | | √ | | |
| TPK | | √ | | | | | | | | √ | | |
| hsCRP (also outcome measure) | | √ | | | | | | | | √ | | |
| U-hCG | | √ | √ | | | | | | | √ | | |
| U-tox | | √ | √ | | | | | | √ | √ | | |
| MADRS | | √ | | | √ | | | | | √ | | |

| Study medication distribution and accountability | | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 | Visit 9 | FU +7 | FU +30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study medication distribution | | | √ | √ | √ | √ | √ | √ | √ | | | |
| Drug accountability, pill count | | | | √ | √ | √ | √ | √ | √ | √ | | |
| Drug plasma concentration BUP | | | | | √ | | √ | | | | | |
| Drug plasma concentration VAR | | | | | √ | | √ | | | | | |

| Separate Study (Pharmacogentics) | | Scr | Day 1 | Day 1 | Day 1 | Day 1 | Day 1 | Day 1 | Day 1 | Day 1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blood sampling for biobanking (once) | | | (√) | (√) | (√) | (√) | (√) | (√) | (√) | (√) | | |

# Appendix D. **Pill count**

The randomized investigational medicinal product (IMP) is distributed as IMP 1 and IMP 2, where each subjects receive both IMP 1 and IMP 2. The first week of the treatment subjects are intended to take 11 doses (distributed in capsules at visit two) of IMP 1 and seven doses of IMP 2. The following visits 35 capsules are distributed (per IMP), where 28 capsules are intended to be ingested in the following 14 days and the seven remaining capsules are to be returned. The extra seven capsules are to be used if the number of days to the next visit exceed 14 days (to prevent subjects going without IMP during the treatment period). No extra capsules are distributed for the first week since it is defined as the dose escalation period.

The pill count estimates the total number of ingested and returned capsules corresponding to have taken the IMP 80% or 100% of the treatment period. At least 80% for both IMP 1 and IMP 2 must be meet, and it corresponds with a maximum of 78 returned capsules for IMP 1 and 77 returned capsules for IMP 2. The pill count calculations for the whole study period are shown below.

**IMP 1**

Total distribution of IMP 1: (35x6+11) = 221

IMP 1 100% pill count: (28x6+11) = 179 (returned 42)

IMP 1 80% pill count: (179*0,8) = 143,2 ≈ 143 (returned 78)

**IMP 2**

Total distribution of IMP 2: (35x6+7) = 217

IMP 2 100% pill count: (28x6+7) = 175 (returned 42)

IMP 2 80% pill count: (175*0,8) = 140 (returned 77)

Table 5. Summary of distributed IMP.

| IMP | Kit | Visit | Distributed number of capsules | Number of days to next visit |
|---|---|---|---|---|
| 1 | 1 | 2 | 11 | 7 |
| 1 | 2 | 3 | 28+7 | 14 |
| 1 | 3 | 4 | 28+7 | 14 |
| 1 | 4 | 5 | 28+7 | 14 |
| 1 | 5 | 6 | 28+7 | 14 |
| 1 | 6 | 7 | 28+7 | 14 |
| 1 | 7 | 8 | 28+7 | 14 |
| 2 | 1 | 2 | 7 | 7 |
| 2 | 2 | 3 | 28+7 | 14 |
| 2 | 3 | 4 | 28+7 | 14 |
| 2 | 4 | 5 | 28+7 | 14 |
| 2 | 5 | 6 | 28+7 | 14 |
| 2 | 6 | 7 | 28+7 | 14 |
| 2 | 7 | 8 | 28+7 | 14 |

Table 6. Pill count for 80% or 100% correct ingested and returned capsules of IMP.

| IMP | Total number of capsules distributed | Total number of days | 80% pill count: ingested capsules (returned ) | 100% pill count: ingested capsules (returned ) |
|---|---|---|---|---|
| 1 | 221 | 91 | 143 (78) | 179 (42) |
| 2 | 217 | 91 | 140 (77) | 175 (42) |

# Appendix E. Baseline characteristics table

The table should be stratified by treatment arm, with one column for each arm.

Percentages should be rounded to whole numbers, means and SDs to two significant digits.

Below is a list of characteristics to be included the table.

**Endpoints**

- Baseline PEth
- Baseline HDD

**Demographics**

- Gender
- Age

Civil status

- Education (summarized as grundskola, gymnasium, universitet<=3, universitet>3, other)

Body weight

**Alcohol related**

- Age of debut
- Family history of alcohol abuse

# Verifikat

Transaktion 09222115557501172295

## Dokument

COMB SAP amendment 230913B
Huvuddokument
29 sidor
*Startades 2023-09-22 08:37:29 CEST (+0200) av Magnus Pettersson (MP)*
*Färdigställt 2023-11-13 20:04:01 CET (+0100)*

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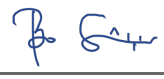

*Signerade 2023-11-13 20:04:01 CET (+0100)*