STATISTICAL ANALYSIS PLAN

Investigation of the Efficacy of Acamprosate and Calcium in Comparison to Placebo as Validation of a Behavioural Test for Alcohol Dependence (TEMACA)

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Signatures

The following persons agree to the content of the statistical analysis plan and indicate this with their signature.

Study management

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1 STUDY BACKGROUND

1.1 STUDY OBJECTIVES

The main research question is whether the current "Behavioral Test for the Development of medications for Alcohol Dependence" (TEMA) can demonstrate that the administration of acamprosate or ionized Calcium (Ca^{2+} = finished drug Calcium-Verla®) reduces the willingness to work for alcohol in a laboratory experiment following a 15- to 20-day period of alcohol abstinence.

The willingness to work for alcohol is determined by the cumulative number of work trials in the "constant attention task" (CAT). The change in this willingness due to the administration of acamprosate, Ca^{2+}, or placebo is expressed as the difference in the cumulative number of work trials in the CAT per test day between Visit 2 (without medication) and Visit 5 (after administration of scamprosate, Ca^{2+} or placebo).

The hypothesis to be tested is that the administration of acamprosate compared to placebo or the administration of calcium ions compared to placebo, leads to a significant reduction in the willingness to work in the laboratory experiment to obtain alcohol.

Based on literature reports that form the basis of the sample size calculation, a relevant change is considered to be a reduction in blood alcohol concentration (BAC) of at least 0.1 per mille in one of the active treatment groups between Visit 2 and Visit 5 compared to the placebo group. In terms of the primary endpoint, this corresponds to a reduction in the cumulative number of work trials for alcohol infusions by 60.

Secondary objectives

The study aims to investigate whether:

1. the administration of acamprosate or calcium ions (Ca^{2+} = ready-to-use drug Calcium-Verla®) compared to placebo leads to altered subjective perception of alcohol effects
2. the efficacy of acamprosate or calcium ions can be predicted by basal levels or the change over time in measures of body calcium reserves reflecting a possible calcium deficiency
3. the administration of acamprosate or calcium ions reduces alcohol craving in daily life
4. the frequency of reported alcohol consumption during the imposed abstinence period differs between the treatment groups and how this affects the willingness to work for alcohol after the imposed abstinence period
5. participation in the study promotes the motivation to change drinking habits or alters drinking habits or the utilization of addiction treatment services
6. the activity of secreted sphingomyelinase is suitable as a biomarker for alcohol consumption and prediction of medication effects
7. safety issues occur during the use of the investigational medicinal products

8. the administration of acamprosate or ionized calcium (Ca^{2+} = ready-to-use drug Calcium-Verla®) lowers threshold at which participants decide it is no longer worthwhile to work for an additional alcohol reward ("break point").
9. the administration of acamprosate or ionized calcium (Ca^{2+} = finished medicinal product Calcium-Verla®) reduces the maximum and mean blood alcohol concentration (BAC) in an alcohol self-administration experiment.
10. the administration of acamprosate or ionized calcium (Ca^{2+} = ready-to-use drug Calcium-Verla®) reduces the work for saline infusions as an alternative reinforcer.
11. the aforementioned points (1 to 3 and 8 to 10) differ in the first and second half of the experiment.
12. the acamprosate level in the group of subjects treated with acamprosate influences the primary outcome of the aforementioned points

1.2 STUDY DESIGN

This is a monocentric study in a placebo-controlled, randomized design with three treatment groups. The administration of the investigational drugs is conducted in a double-blind manner with placebo, acamprosate, or calcium carbonate.

The comparison will be made between the calcium group and the placebo group, as well as between the acamprosate group and the placebo group.

Self-infusion of the NIMP (alcohol / saline solution) will be conducted openly regarding the preparation and single-blind regarding the dosage during visits alcohol self-administration visits V2 and V5.

2 ANALYSIS POPULATIONS

2.1 DEFINITIONS

The intent-to-treat population (ITT) to be analyzed comprises all randomized subjects who were administered the investigational product (excluding the placebo lead-in medication and alcohol) at least once. The statistical analysis of this population determines whether the results are statistically significant or statistically non-significant.

2.2 SERIOUS PROTOCOL VIOLATORS

The per-protocol population (PP) will also be analyzed. This population includes all subjects from the intent-to-treat population who attended visits V5 and V6. Subjects with serious protocol violations will be excluded from the per-protocol analysis. For the analysis of the primary endpoint, these violations include, for example:

- Participants in the acamprosate group with no detectable acamprosate levels in their blood (V5), and

- participants in the calcium group where the temporal profile of body calcium reserve parameters indicates non-compliance with the investigational drug (e.g., a significant drop in calcium levels or an increase in parathyroid hormone).

2.3 FURTHER ANALYSIS

Separate analyses of the ITT and PP populations will be conducted for all subjects who received at least one alcohol infusion during visit 5 (2nd alcohol self-administration). Subjects who only requested saline infusions at visit 5 will not be included in these analyses.

2.4 SAFETY PARAMETERS

The safety parameters will be analyzed using the Safety Analysis Set. This population comprises all subjects who have at least started the administration of an investigational product (including placebo lead-in). The subjects are assigned to the treatment group according to treatment they actually received (analysis "as treated").

3 STUDY CENTERS

This is a monocentric study. The study center is the

Clinic and Polyclinic for Psychiatry and Psychotherapy
University Hospital Carl Gustav Carus
Fetscherstr. 74,
01307 Dresden

4 ANALYSES VARIABLES

4.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

The following data will be recorded: Age in years [AGE], gender [SEX], education level [SCHOOL], training / qualification / degree [QUALI], existing partnership (yes/no) [FAMSTAT; 0=no; 1=yes], living alone (yes/no) [LIFE; 0=no; 1=yes], net household income [INCOME].

Laboratory parameters: Complete blood count [HB, HCT, WBC, PLT, RBC, MCH, MCHC, MCV], ALAT [ALT], ASAT [AST], lipase [LIPA], Crea [CREA], eGFR [eGFROTH], Quick [QUICK], Calcium [CAL], S-ASM [SASM])

Clinical Interviews:

- Diagnostic short Interview for Mental Disorders (Mini-DIPS) [DIPSRES; DIPSSEQ; DIPSTERM]
- Medical assessment of the dependence criteria [SU1 to SU11; 0=no, 1=yes] for alcohol according to DSM-5 and alcohol dependence according to ICD-10 [SUNY = dependence according to ICD 10; 0=no, 1=yes]
- Lifetime Drinking History, including total drinking duration [LtDHDUR], duration of heavy drinking [LtDHHEA], duration of abstinence [LtDHABS], total amount of alcohol consumed [LtDHTOT], average daily alcohol consumption [LtDHDAI]

- Family history using the Family History Assessment Module and Individual Assessment Module (FHAM-IAM) for parents, grandparents and siblings, resulting in a family history [FMHRES; 1=pos; 2=neg] and family density score [FMHFDS].

Questionnaires used:

- Fagerström test for nicotine dependence (FTND) [FTND], including smoking status [SMOKERNY; 1=yes, 0=no]
- Alcohol Use Disorders Identification Test (AUDIT) [AUDIT_QS]
- Alcohol Dependence Scale (ADS) [ADS]
- Barrat Impulsiveness Scale (BIS) [BIS]
- Substance use risk profile scale (SURPS), including Hopelessness [SUPRSH], Anxiety Sensitivity [SUPRSA], Impulsivity [SURPSI], Sensetion Seeking [SURPSS]

Additional laboratory and clinical examinations

- Vital parameters (pulse [PULSE], blood pressure [SYSBP; DIABP]), height [HEIGHT], weight [WEIGHT]

4.2 PRIMARY ENDPOINT

The primary outcome measure is the difference in the cumulative number of work trials for alcohol in the „constant attention task“ (CAT) [CATALC] between the first (V2) and second (V5) self-administration experiment, compared between the acamprosate vs. placebo and calcium vs. placebo treated groups [MED].

4.3 SECONDARY ENDPOINTS

4.3.1 Endpoints related to efficacy

1. Additional parameters of alcohol self-administration:
   a) Difference between visit 2 and visit 5 of the "break points" [BPALC] in the "progressive work" task for alcohol. The "break point" corresponds to the performance requirement at which subjects decide it is no longer worth working for another reward (alcohol) and thus stop working on the CAT before reaching the next reward.
   b) Difference in maximum blood alcohol concentration (BAC) [BACMAX] and mean BAC [BACMEAN] during alcohol self-administration between visits 2 and 5
   c) Difference between visit 2 and 5 in the cumulative number of work cycles for saline [CATWAT] in the "constant attention task"
   d) Analysis of the primary and the above-mentioned secondary parameters of alcohol self-administration with separate consideration of only the first and second half of the experiment respectively. For this purpose, the cumulative number of work cycles for alcohol and saline in the CAT is recorded every 30 seconds during an experiment [CATALC_T0 to CATALC_T300].

2. The subjective alcohol effects will be measured using visual analog scales ("quizzer") before, during and after the experiment at visits 2 and 5 in order to

compare alcohol-induced changes between the 3 treatment arms. The raw values and the "slopes" (increases) between consecutive measurement points will be used for analysis.
The following measures will be collected at 4 points in time (before the experiment, twice during the experiment, once after the experiment):

a) stimulation [VAS1T1, VAS1T2, VAS1T3, VAS1T4],
b) Sedation [VAS2T1, VAS2T2, VAS2T3, VAS2T4]
c) Negative Feelings [VAS3T1, VAS3T2, VAS3T3, VAS3T4]
d) Desire for more alcohol [VAS4T1; VAS4T2; VAS4T3; VAS4T4]
e) Positive feelings [VAS5T1; VAS5T2; VAS5T3; VAS5T4]
f) Drinks [VAS6T1; VAS6T2; VAS6T3; VAS6T4]
g) Feeling drunk [VAS7T1, VAS7T2; VAS7T3; VAS7T4]
h) Thirsty [VAS8T1; VAS8T2; VAS8T3; VAS8T4]

3. Parameters of body calcium reserves will be measured: The blood levels of total calcium [CAL], phosphate [PHOS], magnesium [MG], albumin [ALB], parathyroid hormone [PAHO], 25-hydroxyvitamin D [VITD] at visit 2 and visit 5. Baseline values and the difference in values between V2 and V5 will be considered to examine their relationship to the primary endpoint value in the three treatment arms.
4. Alcohol craving in daily life will be measured using the "Obsessive Compulsive Drinking Scale" (OCDS, from obsessive [OCDSO], compulsive [OCDSK] and total score [OCDST]) before both self-administration experiments (V2 and V5) to compare treatment-related changes between the 3 treatment arms
5. Violations of the imposed abstinence are recorded as the proportion (%) of days with alcohol consumption detected (by TLFB) [VABST] to compare them between the three treatment arms and to investigate their influence on the primary endpoint.
6. Motivation to change drinking habits. The "Readiness to change" questionnaire is used to determine which of the three stages of change in drinking habits the participants are currently assigned to. Subscores of the Pre-Contemplation [RTCP], Contemplation [RTCC] and Action [RTCA] will be anlyzed. Differences between the stage of change before the study (screening), after completion of the study procedures (visit 6) and after completion of the brief intervention (telephone follow-up) will be considered independently of the treatment arm. The number of participants for whom the readiness to change increases, remains the same and decreases will be described.
7. Drinking habits will be assessedusing the Timeline follow-back interview (TLFB) at screening (or re-screening), at visits 1, 3 and 6 and at the telephone follow-up. Changes in drinking habits as a result of study participation will be recorded by comparing the drinking amounts during the 45 days prior to the start of the study (measured at the screening or re-screening) with those after the end of the study (telephone follow-up interview). The following points will be examined in each of the 3 treatment arms:
   a) the percentage of drinking days [TFbITT],
   b) the average amount drunk per drinking day [TFbIAVE],
   c) the percentage of days with heavy alcohol consumption (over 60 g for men and 48 g for women = "binge days") [TFbIBT], and
   d) the average amount of alcohol on these binge days [TFbITM]

8. Determine whether participants have ever received addiction treatment services [SSBNY] (e.g., addiction-specific counseling/treatment by an addiction counseling center or general practitioner or psychologist or psychiatric specialist or hospital) in comparison before study participation (for screening) and afterwards (visit 6 and telephone follow-up interview)
9. Baseline values and change in secreted acid sphingomyelinase activity (at screening [SASM] and at both self-administration experiments, V2 and V5 [SASM1 and SASM2, respectively]) in serum in relation to drinking behavior (TLFB values), primary and secondary endpoints of alcohol self-administration, and medication effects on these outcomes
10. The acamprosate levels [APS] (for the second self-administration experiment, visit 5) in relation to primary and secondary endpoints of alcohol self-administration in the acamprosate group.

4.3.2 Endpoints related to safety/tolerability during the treatment period

1. Occurrence of adverse events from visit V1 to V6 [SAESEQ]
2. CIWA-Ar-Score, values for blood pressure and pulse for screening and visits 1 to 6 [CIWA].

5 MISSING VALUES AND OUTLIERS

5.1 MISSING VALUES

If missing values occur for the primary endpoint (e.g. due to protocol regulations or withdrawal of consent), these will be replaced after reviewing the data using suitable methods.

Additionally, sensitivity analyses will be conducted, including a complete-case analysis (where only participants with all data required for calculating the primary endpoint are analyzed) and, if necessary, data imputation using suitable methods.

5.2 OUTLIERS

A Separate analysis of outliers is not planned.

6 STATISTICAL ANALYSES

6.1 PATIENT AVAILABILITY

The primary analyses will be conducted based on the intention-to-treat population (ITT). Additionally, per-protocol analyses will be conducted as secondary analyses to evaluate the effects of the intervention under ideal conditions.

6.2 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

These data will be analyzed descriptively. For continuous variables such as age, the mean and standard deviation will be calculated. For categorical variables such as gender and partnership status, frequencies and percentages will be calculated.

For laboratory parameters ALAT, ASAT, LIPASE, CREA, eGFR, QUICK and CALCIUM, the central tendencies (mean or median) and the variability (standard deviation or interquartile range) will be calculated. For non-normally distributed data, non-parametric tests will be used for comparison between the subgroups. The normal distribution will be tested using the Kolmogorov-Smirnov test.

The FTND, AUDIT, ADS, BIS and SURPS questionnaires will be analyzed according to their respective scoring guidelines. The resulting scores will be described descriptively with mean and standard deviation and compared between the groups using ANOVA or Kruskal-Wallis test, depending on data distribution.

The vital parameters such as pulse, blood pressure, height and weight will be also analyzed descriptively. Group comparisons, e.g. between sex or age groups, will be conducted using t-tests or Mann-Whitney U-tests, depending on the data distribution; comparisons between treatment groups will be performed using ANOVA or Kruskal-Wallis tests, depending on the data distribution.

6.3 EXPOSURE TO THERAPY AND COMPLIANCE

Randomization will be stratified according to the two criteria:

a) Severity of addiction characteristics up to the time of V4

- "High" if there is at least a moderate alcohol use disorder according to DSM-5, i.e., the presence of at least 4 of the 11 criteria, or if significant physical alcohol withdrawal symptoms were reported anamnestically or observed by study physicians (CIWA at V3 or V4 > 6 points).
- "Low" if there is only a mild alcohol use disorder according to DSM-5, i.e., the presence of no more than 3 of the 11 criteria, and physical withdrawal symptoms were neither reported anamnestically nor observed by physicians..

b) Answer to the question at time V4 as to whether patients want to reduce their alcohol consumption ("yes" or "no")

This stratification aims to ensure that the distribution of these two characteristics does not differ between the three treatment arms before the start of treatment.

It is hypothesized that acamprosate will have a more pronounced effect the more severe the addiction-related pathological changes in the reward system are. This aspect is captured by the composite factor "Severity of Addiction Characteristics" (criterion a).

Since the overwhelming majority of acamprosate studies in humans only included patients who wanted to reduce or stop their alcohol consumption, the clinical efficacy insights of acamprosate are limited to this group. On the other hand, it seems

inappropriate to make the desire for reduction a prerequisite for participation, as based on animal experiments and the observations of Mason et al. (2006), acamprosate reduces alcohol consumption even when there is no explicit desire for change, especially under the specific conditions of prior enforced abstinence.

6.4 CONFIRMATORY ANALYSIS

Testing the two primary hypotheses

H1: The administration of acamprosate compared to placebo leads to a significant reduction in the willingness to perform work in the laboratory experiment to obtain alcohol

H2: The administration of calcium ions compared to placebo leads to a significant reduction in the willingness to perform work in the laboratory experiment to obtain alcohol infusions

Will be conducted using t-tests if the data is normally distributed. The test for normal distribution will be assessed using the Shapiro Wilk test and a graphical inspection of the distribution in the histogram. If the data is not normally distributed, Mann-Whitney tests will be used. The significance level is 0.025, as two null hypotheses are tested in the confirmatory analysis.

6.5 SECONDARY ANALYSES

6.5.1 Efficacy

All parameters listed below will be examined descriptively in a first step and only analyzed using exploratory statistical methods if notable differences or discrepancies are observed.

The aim of exploratory data analysis is to generate hypotheses as a basis for future research projects.

Secondary endpoints related to alcohol self-administration will include:

a. Difference between visit 2 and visit 5 of the "break points" in the "progressive work" scheme for working for alcohol. The "break point" represents the performance threshold at which participants decide it is no longer worth working for another reward (alcohol) and thus stop the CAT before reaching the next reward.

The comparison of the difference in break points between Visit 2 and Visit 5 among the three groups will be conducted descriptively using frequency tables, median, range, maximum, minimum, and appropriate graphical representation.

b. Difference in maximum and mean blood alcohol concentration (BAC) achieved during alcohol self-administration between visits 2 and 5.

This parameter will be analyzed using analyses of variance (ANOVAs).

c. Difference in the cumulative number of work trials for saline solution in the "constant attention task" between visit 2 and visit 5:"

The differences in the three groups will be compared using ANOVA.

d. Investigation of the primary and the above-mentioned secondary parameters of alcohol self-administration, considering only the first and second halves of the experiment. For this purpose, the cumulative number of work trials for alcohol and saline in the CAT will be recorded every 30 seconds during an experiment.

The analyses will be conducted according to the primary and the relevant secondary parameters of alcohol self-administration.

In addition, the following secondary questions will be investigated:

1. does the administration of acamprosate or calcium ions compared to placebo lead to altered subjective perception of alcohol effects?

The subjective perception of alcohol effects will be measured using visual analog scales ("quizzer") before, during and after the experiment at visits 2 and 5 in order to compare their alcohol-induced changes between the 3 treatment arms. The values and the "slopes" (changes) between two measurement points will be analyzed.

Repeated measures ANOVA will be used for analysis, with primary and secondary endpoints of alcohol infusion as covariates. The relationship between alcohol infusion endpoints and subjective data will be further examined using correlation analyse.

2. can the efficacy of acamprosate or calcium ions be predicted by basal levels or changes over time in measures of body calcium reserves indicating possible calcium deficiency?

The blood levels of total calcium, phosphate, magnesium, albumin, parathyroid hormone, 25-hydroxyvitamin D will be measured at visit 2 and visit 5. Baseline values and the difference in values between V2 and V5 will be examined for their relation to the primary endpoint in the three treatment arms.

The relationship between body calcium reserves and efficacy will be investigated using correlation analyses and regression analyses. Group comparisons will be conducted using ANOVA.

3. does the administration of acamprosate or calcium ions reduce alcohol cravings in daily life?

Alcohol craving in daily life will be measured using the "Obsessive Compulsive Drinking Scale" (OCDS) before both self-administration experiments to compare treatment-related changes between the 3 treatment arms.

The change in the total OCDS score and the two subscores will be compared between the three groups using repeated measures ANOVAs.

4. does the frequency of reported alcohol consumption during the imposed abstinence period differ between treatment groups, and how does this affect the work for alcohol after the imposed abstinence period?

Violations of the imposed abstinence will be recorded as the percentage (%) of days with alcohol consumption reported (by TLFB) to compare them between the three treatment arms and to examine their influence on the primary outcome measure.

Group comparisons regarding the frequency of alcohol consumption will be performed using ANOVA. The influence on the primary endpoint will be examined using regression analysis and correlation analysis.

5. does participation in the study promote the motivation to change drinking habits or alter drinking habits or the utilization of addiction treatment services?

Motivation to change drinking habits:

The "Readiness to change" questionnaire will be used to determine which of the three “stages of change” in drinking habits the participants are currently in. Differences in “stages of change” will be assessed at three time points: before study participation (screening), after completion of the study procedures (visit 6) and after completion of the brief intervention (telephone follow-up). These assessments will be considered independently of the treatment arm. The numbers of participants who increase, maintain, or decrease in their stage of change will be recorded.

The stages of change will be analyzed using the Wilcoxon sign-rank test (scaled ordinally).

Changes in the stage of change will be analyzed using ordinal logistic regression.

Drinking habits:

Drinking habits will be assessed using the timeline follow-back interview (TLFB) at screening (or re-screening), at visits 1, 3 and 6 and at the telephone follow-up. Changes in drinking habits due to study participation will be recorded by comparing drinking amounts during the 45 days before the study (measured at the screening or re-screening) with those after the end of the study (telephone follow-up interview). The following points are examined in each of the 3 treatment arms:

a. percentage of drinking days,
b. average amount of alcohol consumed per drinking day,
c. percentage of days with heavy alcohol consumption (over 60 g for men and 48 g for women = "binge days"), and
d. average amount of alcohol consumed on these binge days

Comparisons over time regarding these parameters will be conducted using repeated measures ANOVA.

Utilization of addiction treatment services:

The use of addiction treatment services will be recorded by determining whether the participants have ever accessed any addiction treatment services (e.g. addiction-specific counseling/treatment by an addiction counseling center, general practitioner, psychologist, psychiatrist or hospital) in comparison before study participation (at screening) and afterwards (visit 6 and telephone follow-up survey)

Changes in behavior regarding the use of addiction treatment services will be categorized and analyzed using chi-square tests.

6. is the activity of secreted sphingomyelinase a suitable biomarker for alcohol consumption and prediction of medication effects?

For this purpose, the baseline values and the changes in the activity of secreted acid sphingomyelinase will be measured at screening and both self-administration experiments (V2 and V5) in serum. These will be examined in relation to drinking behavior (TLFB values), primary and secondary endpoints of alcohol self-administration and medication effects.

Analysis will be conducted using correlation analyses.

6.5.2 Safety / tolerability

The CIWA-Ar score will be categorized (mild/moderate/severe) and compared between the treatment groups using a cross-tabulation and chi-square test.

Blood pressure and pulse values will be are documented at screening and visits 1 to 6 and compared using appropriate methods.

6.5.3 Adverse events

The occurrence of adverse events from visit V1 to V6 will be recorded. The frequencies of adverse events in the treatment groups between V1 and V6 will be presented in cross-tabulations; a group comparison will be made using a chi-square test if applicable.

6.5.4 Laboratory parameters

The relationship between activity and efficacy will be examined using correlation analyses and regression analyses. Group comparisons will be performed using ANOVA.

6.5.5 Pharmacokinetics

The relationship between body calcium reserves and efficacy will be is examined using correlation and regression analyses. Group comparisons will be conducted using ANOVA.

The relationship between acamprosate levels in the acamprosate group and efficacy will be analyzed using correlation and regression analyses. Group comparisons will be conducted using ANOVA.

The changes in body calcium reserves will be compared between groups using repeated measures ANOVA.

6.6 SUBGROUP ANALYSES

Exploratory subgroup analyses will be conducted based on gender and the presence of alcohol dependence according to ICD-10 (yes/no) for the parameters mentioned in section 6.

6.7 INTERIM ANALYSES

No interim analyses are planned

7 SOFTWARE

The statistical analyses will be performed using the SPSS software and SAS System for Windows.