EXALT- Statistical Analysis Plan

Does Glucagon-like Peptide 1 (GLP-1) receptor agonist stimulation reduce alcohol intake in patients with alcohol dependence?

EudraCT 2016-003343-11
Clinicaltrials.gov: NCT03232112

SAP date/status	26.05.20/ first edition
Protocol version:	27.04.20/ eight edition

SAP CONTRIBUTORS:

SPONSOR-INVESTIGATOR
Anders Fink-Jensen MD DMSc
Professor
Psychiatric Centre Copenhagen, Rigshospitalet
University of Copenhagen, Denmark

SENIOR STATISTICIAN RESPONSIBLE
Claus Thorn Ekstrøm PhD
Professor
Section of Biostatistics
University of Copenhagen, Denmark

INVESTIGATOR/Performed the SAP
Mette Kruse Klausen MD
Psychiatric Centre Copenhagen, Rigshospitalet
University of Copenhagen, Denmark

Signatures:

Anders Fink-Jensen

_____ Date: 26/5-2020

Claus Thorn Ekstrøm

_____ Date: 26/5-2020

Mette Kruse Klausen

_____ Date: 26/5-20

Abbreviations

AUD	Alcohol Use Disorder
AUDIT	Alcohol use disorder identification test
CBT	Cognitive behavioral therapy
DAT	Dopamine Transporter
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
fMRI	Functional magnetic resonance imaging (brain-scan)
FSL	FMRIB Software Library - Analysis tools for FMRI
GLP1-RA	Glucagon-like peptide-1 receptor agonists
Heavy drinking day	Alcohol consumption over 60/48 g (men/women) of alcohol per day
ICD-10	International Classification of Diseases
PEth	Phosphatidylethanol - Biomarker for alcohol consumption
REDCap	Web application for building and managing online surveys and databases
SPECT	Single-Photon Emission Computed Tomography (brain scan)
SPM	Statistical Parametric Mapping (software for analysis of fMRI data)
TLFB	Time-Line-Follow-Back Method (recording of heavy drinking days)

Table of content

Introduction

Background and rationale

Alcohol use disorder (AUD) is a major public health problem both in Europe and in the United States. AUD is under-diagnosed and undertreated and when alcohol abstinent, more than 2/3 of patients in abstinence-oriented treatment will relapse within the first year. In Denmark, approximately 20% of the population is consuming more alcohol per week than recommended by the Danish Health and Medicines Authority, 14% of the Danish population has a harmful consumption of alcohol and 3% fulfills the criteria for AUD.
One of the best-documented modalities in the treatment of AUD is cognitive-behavioral therapy (CBT). The underlying neuroanatomical basis of alcohol addiction and treatment effects of CBT are not yet established though functional Magnetic Resonance Imaging (fMRI) studies have begun to elucidate the neuroanatomical basis.

The pharmacological treatment of AUD is considered an important supplement to psychological therapy. Disulfiram, a substance that blocks alcohol-metabolizing enzymes resulting in increased acetaldehyde concentrations, has been introduced in Denmark many years ago and is still the most used drug against AUD in Denmark. Newer pharmacological agents as acamprosate and naltrexone have been introduced to the clinic. However, these compounds have not, despite numerous randomised clinical trials (RCT), gained widespread dissemination, probably because the effect of these substances has been modest, with a less than 10% increase in abstinent rate compared to placebo.

As the success rates of CBT are moderate and the synergistic effects of adding pharmacological treatment – as described above – are quite limited, strong needs still exist for efficient, molecular targets in the medical treatment of alcohol dependence.

GLP-1 based therapy was introduced to the market for the treatment of type 2 diabetes in 2006. GLP-1 is an incretin hormone, which is secreted from endocrine L cells of the small intestine in response to nutrients in the gut lumen. Exendin-4, originally isolated from the saliva of a lizard species, the Gila monster, has 53% sequence homology with human GLP-1 in its first 30 amino acids. Synthetic exendin-4, referred to as exenatide, is resistant to DPP-4 cleavage and therefore has a significantly longer half-life which makes it useful for the treatment of type 2 diabetes.

GLP-1RA has a well-established effect on food reward which seems to be driven by two key mesolimbic brain structures, the ventral tegmental area (VTA) and nucleus accumbens (NAc). These structures are not only involved in the rewarding properties of food but also drugs of abuse, including alcohol. A link between alcohol intake and GLP-1 has been demonstrated in studies, as alcohol intake can result in an elevated level of gut-produced GLP-1 in rats. Elevation of dopamine levels in brain striatum following stimulant administration has been demonstrated in multiple clinical trials and dopamine is considered to have a central role in addiction to stimulant drugs (i.e. amphetamine, alcohol). Preclinical studies have demonstrated the inhibitory effects of the GLP-1RA exendin-4 on alcohol-mediated behavior in rodents and

non-human primates. The precise mechanism of action has not been elucidated; however, we have recently reported that exendin-4 induced upregulation of the dopamine transporter (DAT) function in vitro.

GLP-1 analogs: A potential new treatment for alcohol use disorder?
Based on the identified medical need for novel molecular targets in the medical treatment of AUD and the promising data of GLP-1R stimulation in alcohol-mediated behavior in rodents, non-human primates and patients with type 2 diabetes, we aim to investigate whether these beneficial findings on alcohol consumption can be extended to encompass patients with known alcohol use disorder.

Objectives

- Treatment with exenatide will decrease alcohol consumption, measured as the total number of heavy drinking days, for the last 30 days, measured by the Time-Line-Follow-Back-Method, in patients with alcohol use disorder.
- Exenatide will induce upregulation of the striatal DAT availability, in patients with alcohol use disorder quantified by SPECT-scans.
- Exenatide will modulate neural responses in reward processing brain regions including NAc quantified by fMRI-scans.

Study Methods

Trial design

The EXALT trial[1] was a randomised, double-blinded, placebo-controlled clinical trial for 26 weeks and a long term follow up 26 weeks after the end of participation in the main trial. Comparative treatment regimens were once-weekly injections with exenatide 2 mg (Bydureon®) or placebo (saline) as a supplement to cognitive behavioral therapy. Baseline data were collected at the screening session. Follow-up meetings were scheduled at weeks 4, 12, 20, and a final evaluation at week 26.
A subgroup of patients had a baseline SPECT- and an fMRI scan or just an fMRI scan performed before receiving 1. injection, and again when they finalized the study.
A group of 25 individuals matched with age, gender, education, and no history of alcohol use disorder had the same screening blood-samples and the same fMRI scan performed.

Randomization

The participants were randomised in two groups, exanetide or placebo. The randomisation was stratified in terms of age, sex, and baseline alcohol consumption i.e. the number of heavy drinking days in the last 30 days before inclusion measured by the TLFB-method (5-11, 12-17, 18-23, 24-30 days). For this stratified randomisation, the randomisation tool in REDCap was used. Patients, investigators, other caregivers performing assessments, and persons performing data analysis have been blinded from the time of randomisation and until the time of database unlock. To maintain the blinding, the participants were blindfolded and an un-blinded nurse prepared the injections in a separate room and gave the injection immediately after.

Sample size

The original sample size calculation was performed with an estimated drop-out rate of 40 % and a power of 90%. That led to a total number of 114 patients to include. Please see appendix 1. In May 2019 when recruitment was almost finished, the dropout rate was nearly 60%. We choose as a consequence to do a

new power calculation with a drop out rate of 60% and a power of 85% (please see below). Due to a lack of patients to be included we stopped recruitment 1st of October 2019.

Justification of sample size incl. power calculation

The primary outcome measure, i.e. the total number of heavy drinking days, was used for the sample size calculation. Based on data from the study by Johnson et al[2], where the reduction in the percentage points of the total number of heavy drinking days was 60 pp in the intervention group and 33 pp in the control group. With an alpha of 5 %, and a power of 85 %, and with an estimated SD of 34.5, the estimated sample size was of 58 patients (29 in each group). With an estimated dropout rate of 60 % a total number of 144 patients (72 patients in each arm) was needed (please see below)

Heavy drinking days:
Reduction in heavy drinking days: Topiramate versus placebo: 60.34 contra 32.73
SD estimate 34.5

The POWER Procedure
Two-sample t Test for Mean Difference
Fixed Scenario Elements

Distribution	Normal
Method	Exact
Group 1 Mean	60.34
Group 2 Mean	32.73
Standard Deviation	34.5
Number of Sides	2
Null Difference	0
Alpha	0.05

Computed N per group

Nominal Index	Actual Power	N Power	Per Group
1	0.85	0.85	29

Framework, Statistical interim analysis and stopping guidance

No superiority, equivalence, or noninferiority hypothesis testing framework will be performed.
No interim analysis was performed.

Timing of final analysis

All analyses will be carried out with the treatment groups still blinded and labeled as "treatment group A" and "treatment group B". Before dividing participants into group A and group B, the statistical analysis plan will be completed, signed, and uploaded at clinicaltrials.gov, and the data set locked. The final unblinding of treatment groups (exanetide or placebo), will not be carried out until all statistical analyses are performed.

All data besides data from the long term follow up, will be analysed collectively. The last long-term follow up-visit is in September 2020, and the final data will be analysed unblinded

Timing of outcome assessments

All visits are planned from the date of the first injection and are performed + one week (i.e. week 5, 13, 21, 27). All irregularities are recorded as protocol deviations and visit "windows" are within 2 weeks (+/-).

Statistical Principles

Confidence and P-values

The level of statistical significance will be with an alpha set at 0.05, two-sided testing, and a confidence interval of 95%. There will be no adjustment for multiplicity.

Adherence and protocol deviations

Adherence to the intervention was defined by no more than 3 missed consecutive injections, or 5 missed injections in total during the 26-week period. The un-blinded project nurses kept an individual injection schedule per patient and thereby kept track of the extent of exposure. Adherence to the intervention will be presented as a table of distribution of percentage of weeks with study medication injections in the main treatment period. Protocol deviations will be presented in a table divided into the categories: study procedure, eligibility, and randomisation. Only protocol deviations regarding "lost to follow up" and "withdrawal" will be summarized in the section named this.

Analysis populations

All analyses will be performed using the intention-to-treat principle on subjects, who were randomized and received at least one dose of the trial compound (Bydureon® or placebo).

Trial Population

Screening data

Screening data will be presented in the CONSORT flowchart

Eligibility

Individuals aged 18-70 diagnosed with AUD according to ICD 10 and DSM-5 with an AUDIT score >15 and with at least 5 heavy drinking days in the past 30 days. Furthermore, no severe psychiatric-, neurological- or somatic disease, history of alcohol withdrawal seizures, diabetes, other substance use disorder, or concomitant pharmacotherapy against alcohol use disorder.

Besides the patients described above, 25 healthy controls matched with sex, age, and education and with no record of AUD were recruited to perform the same fMRI-scans as the patients included in the study.

Recruitment

Recruitment began on the 1st of August 2017 an ended 1st of October 2019. The last patient visit in the main study was planned to be the 8th of April 2020, but because of the COVID-19 outbreak, all patients were terminated 14th of March 2020. The final long-term follow up, will take place in September 2020. All participants were recruited from the suburbs of Copenhagen, Denmark.

Withdrawal/Follow up

All patients who have withdrawn from the intervention and have been included for at least eight weeks (steady-state plasma level) were encouraged to participate in the last week 26 follow-up including brain scans at the time of withdrawal. If successful, individuals will be contacted for a long-term follow-up after further 26 weeks. All other individuals e.g individuals who withdraw before eight weeks and lost to follow-up will not be contacted for a long-term follow-up. Data will be presented in a Kaplan Meyer survival curve

Baseline Patient characteristics

List of baseline characteristics to be summarised:

- Sex
- Age mean
- Age group (+/- 40 years)
- Education (lower secondary school, upper secondary school, vocational education + short-cycle higher education, medium cycle higher education + higher education)
- Marital status (yes, no)
- Job-status (yes, no)
- Heavy days, mean
- Heavy days group (5-11, 12-17, 18-23, 24-30)
- Baseline AUDIT score, mean
- Baseline AUDIT group (15-19, 20-40)
- 0-days during the prior 30 days to inclusion, mean
- Total alcohol consumption during the prior 30 days to inclusion, mean
- ICD-10 number of diagnostic items (4,5,6)
- DSM-5 group (mild(2-3), moderate(4-5), severe(>6))

Baseline characteristics will be descriptively summarized in a table assessed by the intervention

Analysis

Outcome definitions

Primary endpoint

Percentage of heavy drinking days during the last 30 days, at follow-up after 26 weeks of treatment adjusted for baseline (percentage points (pp)). A heavy-drinking day is defined as a day with an excess intake of 60/48 grams (men/women) of alcohol per day. Data will be registered via the Timeline-Follow-Back-method (TLFB).

Secondary endpoints

1. Total alcohol consumption (gram/day) during the last 30 days, at follow up after 26 weeks of treatment adjusted for baseline. Data will be registered via the Timeline-Follow-Back-method (TLFB).
2. Percentage of days without any alcohol consumption during the last 30 days, at follow-up after 26 weeks of treatment adjusted for baseline (percentage points (pp)). Data will be registered via the Timeline-Follow-Back-method (TLFB).
3. Phosphatidylethanol (PEth) (ng/mL) at follow-up after 26 weeks of treatment, adjusted for baseline.
4. Penn Alcohol Craving Scale (PACS) score at follow-up after 26 weeks of treatment, adjusted for baseline.

5. Alcohol Use Disorders Identification Test (AUDIT) score at follow-up after 26 weeks of treatment, adjusted for baseline.
6. Screen for Cognitive Impairment in Psychiatry test (SCIP) score at follow-up after 26 weeks of treatment, adjusted for baseline and week 4.
7. The liver parameter alanine aminotransferase (ALAT) (U/L) at follow up after 26 weeks of treatment, adjusted for baseline.
8. The liver parameter gamma-glutamyl transferase (GGT) (U/L) at follow up after 26 weeks of treatment, adjusted for baseline.
9. Mean cell volume (MCV) (fL) at follow up after 26 weeks of treatment, adjusted for baseline.
10. Short-Form Health Survey (SF-36) score at follow-up after 26 weeks of treatment, adjusted for baseline.
11. Symptom Checklist (SCL-92) score at follow-up after 26 weeks of treatment, adjusted for baseline.
12. Bodyweight (kg) at follow-up after 26 weeks of treatment, adjusted for baseline.
13. Blood pressure (mmHg) at follow-up after 26 weeks of treatment, adjusted for baseline.
14. Pulse (PR) at follow-up after 26 weeks of treatment, adjusted for baseline.
15. Waist circumference (cm) at follow-up after 26 weeks of treatment, adjusted for baseline.
16. Glycemic control parameters - HbA1c (mmol/L) at follow-up after 26 weeks of treatment, adjusted for baseline.
17. Kidney function (urine albumin/creatinine ratio) at follow-up after 26 weeks of treatment, adjusted for baseline.
18. Drug Use Disorders Identification Test (DUDIT) score at follow-up after 26 weeks of treatment, adjusted for baseline.
19. Percentage of heavy drinking days during the last 30 days, at the long term follow up 26 weeks after termination of intervention, adjusted for baseline and end of treatment (week 26) (percentage points (pp)).

SPECT:

20. Specific Binding Ratio (SBR) in the striatum, putamen, and caudatus from baseline SPECT-scan to follow-up after 26 weeks of treatment, adjusted for baseline.

fMRI:

21. Blood oxygenation level-dependent (BOLD) percent signal change (contrast "alcohol vs. neutral cues") in the ventral striatum (nucleus accumbens), the putamen and the caudatus. Group (exanetide or placebo), session (baseline or follow-up scan), and patients vs healthy control-group.
22. Craving data acquired during fMRI scan. Group (exanetide or placebo), session (baseline or follow-up scan), category (alcohol or neutral stimuli) and patient vs healthy control-group.

Analysis methods

All continuous outcomes, i.e., change in metabolic parameters, weight, body composition parameters, alcohol use, etc. will be analyzed using ANOVA from baseline to last observation endpoint. Categorical outcomes will be analyzed using chi-square analyses. As the study was conducted as a randomized trial, no covariates adjustment is in principle necessary to assess causal effects.

We will use residual plots and qq-plots in combination with Wally plots[3] to validate the normality assumptions of the residuals in the ANOVA models. If the distributional assumption about normality of the residuals does not hold then we will first see if a log-transform of the outcome will result in a model fit that does not appear to violate the assumptions of normality. If the log-transform still does not hold, our next step will be to consider if the lack of fit is caused by inflation, e.g. due to too many observations being zero. Should that be the case we will replace the standard ANOVA model with a zero-inflated gaussian mixture model for the analyses. Should none of these approaches work we will use quantile regression and model the median outcome instead.

Sensitivity analysis

Sensitivity analysis is planned for the outcomes: heavy days, total alcohol intake, 0-days with the following assumptions:

1. *We assume the drop-out is caused by a relapse in alcohol intake, and therefore we will impute the values of the missing final data for the above categories, as the baseline value and half a baseline-value.*
2. *We assume the missing data are MNAR and will make a per protocol (PP) analysis.*

We assume that the amount of chlordiazepoxide at screening will influence the baseline SCIP and PACS-score. We will do a sensitivity analysis and compare the SCIP-results from week 4 with the baseline results, to see if there is a difference.

Subgroup analysis

We will perform the following subgroup analysis:

- Baseline heavy days (5-11, 12-17, 18-23, 24-30)
- DSM-5 group (mild(2-3), moderate(4-5), severe(>6))
- Geography (Hvidovre, Lyngby, Glostrup, Koege)

Posthoc analysis

- SPECT-scan: The difference in baseline SBR (Striatum, Putamen, Caudatus) with a healthy control sample.
- Data from the 6 months follow-up (PACS, PEth, AUDIT, Fagerstroems for nicotine)
- Plasma exanetide concentration level and antibody-level related to heavy drinking days
- Plasma FGF-21 levels
- Urine oxidative stress parameters
- Plasma bone markers (TRAP-5b, CTX, PINP)
- Proteomic fingerprint
- fMRI Spatial working memory (N-back task)

Missing data

Missing data will be imputed using the multiple imputation method. However, for multiple imputations to work properly we need to assume that the data are missing at random (MAR) which may not be the case in our study. Consequently, as a sensitivity analysis of handling the missing data, we will consider two realistic alternatives for the missing mechanism: 1) to assume that all non-observed individuals at 26 weeks have reverted to their pre-study drinking habit, and 2) that all non-observed individuals at 26 weeks have halved

their drinking. If only multiple outcomes are missing then we will use a linear mixed-effect model which is an extension of the ANOVA model for analyzing the data.

Additional analysis

Our primary analysis compares treated with untreated in an intention-to-treat (ITT) setup which provides valid estimates of the real-world effects. To get an estimate of the complier-average causal effect we will use information from the questionnaire of the drop-outs using the principal stratification approach of Frangakis and Robin[4]

Harms

Safety data has been collected in the 26 weeks of inclusion and up until 10 weeks after termination of the study for all individuals. Data has been classified as Serious Adverse Events (SAE), Adverse Events (AE), and Adverse Reactions (AR). All collected safety data will be summarized in a table with incidence cases.

Statistical software

All statistical analysis will be performed with RStudio[5]. fMRI data will be analyzed with the statistical software SPM and FSL. The SPECT data is quantified with DATquan which is a Matlab-based clinical software tool that can provide fast, accurate, and highly reproducible quantification of DAT binding in brain SPECT images.

References

1. Antonsen KK, Klausen MK, Brunchmann AS, et al. Does glucagon-like peptide-1 (GLP-1) receptor agonist stimulation reduce alcohol intake in patients with alcohol dependence: study protocol of a randomised, double-blinded, placebo-controlled clinical trial. BMJ Open 2018;8(7):e019562.
2. Johnson BA, Ait-Daoud N, Bowden CL, et al. Oral topiramate for treatment of alcohol dependence: A randomised controlled trial. Lancet 2003;361(9370):1677–85.
3. Ekstrøm CT. Teaching "Instant Experience" with Graphical Model Validation Techniques. Teach Stat 2014;36(1):23–6.
4. Frangakis CE, Rubin DB. Principal stratification in causal inference. Biometrics 2002;58(1):21–9.
5. RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/

Appendix 1

Justification of sample size incl. power calculation

The primary outcome measure (total number of heavy drinking days) was used for the sample size calculation. Based on data from the study by Johnson et al[2], where the reduction in the percentage points of the total number of heavy drinking days was 60 pp in the intervention group and 33 pp in the control group, with an alpha of 5 %, and a power of 90 %, and with an estimated SD of 34.5 pp, the estimated sample size is of 68 patients (34 in each group). With an estimated dropout rate of 40 % a total number of 114 patients (57 patients in each arm) is needed (please see below)

Heavy drinking days:

Reduction in heavy drinking days: Topiramate versus placebo: 60.34 contra 32.73

SD estimate 34.5

The POWER Procedure

Two-sample t Test for Mean Difference

Fixed Scenario Elements

Distribution	Normal
Method	Exact
Group 1 Mean	60.34
Group 2 Mean	32.73
Standard Deviation	34.5
Number of Sides	2
Null Difference	0
Alpha	0.05

Computed N per group

Nominal Index	Actual Power	N Power	Per Group
1	0.9	0.902	34