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Does glucagon-like peptide-1 (GLP-1) receptor stimulation reduce alcohol intake in patients with alcohol dependence? A randomized, double-blinded, placebo-controlled clinical trial

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Abstract

Introduction: Alcohol dependence is a major public health problem. It is under-diagnosed and undertreated. Even when treated, more than 2/3 of patients in abstinence-oriented treatment will relapse within the first year. Thus, there is an urgent need for efficacious medical treatment of alcohol dependence. Glucagon-like peptide-1 (GLP-1) receptor stimulation has proven to reduce alcohol consumption in preclinical experiments. However, the effect of GLP-1 receptor agonists in humans has to our knowledge, not yet been investigated. **Methods and analysis**: *Design, participants and intervention*: The effect of the once-weekly GLP-1-receptor-agonist, exenatide will be investigated in a double-blinded, placebo-controlled, randomized clinical trial. One hundred and fourteen outpatients will be recruited and randomized to treatment with either placebo or exenatide once-weekly for 26 weeks as a supplement to cognitive behavioural therapy. *The primary endpoint* is reduction in number of 'heavy drinking days'. *The secondary endpoints* include changes in total alcohol consumption, days without consumption, changes in brain activity and function, smoking status, cognition, measures of quality of life and changes in phosphatidylethanol (PEth) as a biomarker of alcohol consumption from baseline to follow-up at week 26. *Status*: Currently recruiting patients. **Ethics and dissemination**: Ethical approval has been obtained. Before screening, all patients will be provided oral and written information about the trial. The study results will be disseminated by peer-review publications and conference presentations and has the potential to reveal a completely new medical treatment of alcohol dependence.

Strengths and limitations of this study

- The study design, i.e. a double-blinded, randomized, placebo-controlled clinical trial, is a strength as it is designed to evaluate the effects of the GLP-1 receptor agonist exenatide on alcohol consumption in patients with alcohol dependence
- The study duration is 26 weeks which is longer than most previous studies investigating medical treatment of alcohol dependence
- The biological basis for any demonstrated effect is investigated with brain imaging techniques and the biomarker phosphatidylethanol (PEth)
- A possible limitation is that the alcohol intake is self-reported which potentially could affect accuracy
- Another limitation is that the study has no third treatment arm for comparing exenatide to one of the known compounds used in the clinic against alcohol use disorder, e.g. disulfiram, acamprosate or naltrexone.

Introduction

Alcohol dependence is a major global public health problem across the world[1,2]. It is an under-diagnosed and undertreated[3] condition and more than 2/3 of patients in abstinence-oriented treatment will relapse within the first year[4]. In Denmark, approximately 20% of the population is consuming more alcohol than recommended by the Danish National Board of Health[5]. Further, 8.5% of the Danish adult population (16 years or older) has a 'risky', i.e. potentially harmful, alcohol consumption, defined as more than 14 and 21 units of alcohol (one unit defined as 12 grams of pure alcohol) for women and men per week, respectively[6]. Three percent fulfils the criteria for alcohol dependence[7].

Psychological treatment

One of the best documented treatments of alcohol dependence is cognitive behavioural therapy (CBT)[8]. The underlying neuroanatomical basis of alcohol addiction and treatment effects of CBT are not yet established, although functional Magnetic Resonance Imaging (fMRI) studies have begun to elucidate the neural underpinnings of alcohol dependence[9]. Alcohol dependent patients have been found to display increased dorsal anterior cingulate cortex (dACC) activation during spatial working memory, perhaps reflecting decreased prefrontal efficiency because of distracting alcohol related thoughts[10]. Interestingly, it was also recently demonstrated that alcohol dependent patients also display increased neural activation to alcohol associated cues in mesocortico-limbic networks; which is normalized with psychological therapy[9].

Pharmacological treatment

Pharmacological treatment of alcohol dependence is considered an important supplement to psychological therapy[11]. *Disulfiram*, a substance that blocks alcohol-metabolizing enzymes resulting in increased acetaldehyde concentrations, was introduced in Denmark in 1948[12]. It is still the most frequently used drug for treatment of alcohol dependence in Denmark[11] although the evidence for its effect is not that strong[11]. Newer pharmacological agents such as *Acamprosate* a gamma-amino-butyric-acid (GABA) receptor agonist and the glutamate N-Methyl-D-aspartate (NMDA) receptor antagonist; and *naltrexone*, a mu and kappa opioid receptor antagonist are now used as alternate treatments for alcohol dependence. However, these compounds have not gained widespread dissemination, probably because the effect of the substances is modest, with a less than 10% increase in abstinent rate compared to placebo[13]. The antiepileptic compound *topiramate* has shown promising results in clinical trials[14] and another pharmacological agent, *nalmefene*,

with a mechanism of action somewhat similar to naltrexone, has very recently been approved by the European Medicines Agency (EMA) as a medication for reducing alcohol consumption [15]. Clearly, given the moderate success rates of CBT[8] and the synergistic effects of adding pharmacological treatment – as described above – are quite limited, there is an urgent need for new and more efficient treatment modalities of alcohol dependence.

Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists (GLP-1RA)

GLP-1 based therapy for the treatment of type 2 diabetes was introduced in 2006[16]. 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[17]. GLP-1 conveys an insulinotropic effect through GLP-1 receptors (GLP-1R) on the beta cells of the pancreas and inhibits the secretion of glucagon from the alpha cells of the pancreas, which lower the blood glucose level[18]. Thus, GLP-1 is central for glycaemic control. Importantly, these effects are strictly glucose-dependent (more pronounced at higher levels of blood glucose) as the effect ceases when the blood glucose reaches values below 4-5 moll/L[17]. Naturally occurring GLP-1 is rapidly degraded within minutes by the enzyme, dipeptidyl peptidase 4 (DPP-4)[17]. 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[16]. Exenatide binds to the GLP-1R with high affinity[19] and acts as a receptor agonist, thus referred to as a GLP-1RA.

GLP-1RA: A potential new treatment for alcohol use disorder?

GLP-1RA has a well-established effect on the food reward system which seems to be driven by two key mesolimbic brain regions, the ventral tegmental area (VTA) and nucleus accumbens (NAc)[20]. These regions are not only involved in the rewarding properties of food but also to drugs of abuse, including alcohol[21,22,23]. Interestingly, GLP-1 receptors (GLP-1R) are expressed in these brain reward regions (VTA and NAc), which are innervated by hindbrain GLP-1 neurons[21]. A link between alcohol intake and GLP-1 has been demonstrated in studies demonstrating that alcohol intake can result in an elevated level of gut-produced GLP-1 in rats[22]. Elevation of dopamine levels in NAc following drugs of abuse has been demonstrated in multiple preclinical and clinical studies and is considered to play a central role in development of addiction to stimulant drugs (i.e. cocaine, amphetamine, alcohol)[24]. Preclinical studies demonstrated the inhibitory effects of the GLP-1RA exendin-4 on alcohol-mediated behaviour in rodents and in another study systemic administration of exendin-4 reduced alcohol-induced dopamine release in the NAc[21]. These findings are consistent with the hypothesis, that systemic administration of GLP-1RA can influence the mesolimbic dopamine system and reward-seeking behaviours associated with alcohol dependence[25]. Although the precise mechanism of action has not been elucidated *in vivo*, we recently reported that *in vitro*, exendin-4 induces an upregulation of the dopamine transporter (DAT) function[26].

Given this collective evidence, we aim to investigate whether the beneficial effect of the GLP-1 receptor agonist, exenatide, on alcohol consumption in preclinical studies, can be translated to patients with known alcohol use disorder[27].

Hypothesis

- Exenatide treatment will decrease alcohol consumption, measured as total number of heavy drinking days, in alcohol dependent patients.

- Exenatide will induce upregulation of the striatal dopamine transporter availability, in alcohol dependent patients.
- Exenatide will modulate neural responses in reward processing regions including nucleus accumbens.

To test these hypothesis, we have designed a 26-week, clinical trial including 114 patients with known alcohol dependence. To explore the underlying neuromolecular mechanism(s) of the potential positive effect of exenatide vs. placebo on alcohol consumption, we will obtain Single-Photon Emission Computed Tomography (SPECT) neuroimaging of DAT at week 0 and 26 in a subgroup of the patients. Further, the functional brain network modulated by the possible treatment effects will be investigated using fMRI at week 0 and 26.

Methods and analysis

Study design

The present study is a 26-week, double-blinded, randomized, placebo-controlled clinical trial, designed to evaluate the effects of exenatide vs. placebo in 114 patients, diagnosed with alcohol dependence according to the International Classification of Diseases, tenth edition (ICD-10) and with an alcohol use disorder according to the The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria. The patients will be recruited from the Novavì outpatient clinics in Copenhagen, Denmark. To be eligible for participation, the patients will first undergo screening according to the inclusion and exclusion criteria. When included and consented, the patient will meet to get his/her weekly injection by the un-blinded nurse. The nurse will collect a weekly alcohol diary and hand out a new one, for the following week.

Participants and screening

The patients will be recruited from outpatient units, specifically, the Novavì outpatient clinics in suburbs of Copenhagen, Denmark. All patients will receive psychosocial alcohol treatment based on psycho educative elements, motivational interviewing and CBT. Skilled staff members will be administering the psychosocial treatment in the clinic. The recruitment procedure starts as a pre-screening when the patients contact the Novavì outpatient clinics, which are open outpatient clinics. All potentially eligible patients will be fully informed, verbally as well as in writing, of their rights and responsibilities while participating in the trial. Screening examinations will only be performed after the patient has agreed to participate and has signed the informed consent form.

At the time of screening the patients will undergo a series of examinations to assure that all in- and exclusion criteria are met. The patients will be asked general information about psychosocial factors, i.e., education level, employment- and marital status. In addition, somatic symptoms and baseline medications will be registered. Blood samples and a urine tests will be collected for acute analysis according to the exclusion criteria. Furthermore, blood- and urine samples will be saved for an investigational biobank that will allow more advanced analyses, e.g. phosphatidylethanol (PEth). The most important tests and examinations in the study are described in details in later sections. See also the *figure 1* for a complete schedule of events.

Intervention

The pharmacological intervention will be given as an add-on to the standardized psychosocial alcohol treatment paradigm. Exenatide is delivered from *Region Hovedstadens Apotek* as a powder with solvent for

prolonged release injection (once-weekly). Each single-dose, dual-chamber pen contains 0.65 ml of diluent and 2 mg of exenatide, which are isolated until mixed by the nurse administering the drug. The placebo will be supplied as pre-filled saline syringes (0.9% saline), by *Region Hovedstadens Apotek* and will be administered in the same way and volume as exenatide. The un-blinded nurse, with no involvement in the psychosocial treatment, will administer the drug injections, and the patients will be blindfolded while receiving the once-weekly injections.

Inclusion criteria

- Informed oral and written consent
- Diagnosed with alcohol dependence according to the criteria of ICD-10, World Health Organization and DSM-5 (for the equivalent diagnosis of alcohol use disorder)
- Alcohol use disorder identification test (AUDIT) score >15[28]
- Age 18 - 70 years (both included)
- Heavy alcohol drinking defined as having alcohol consumption over 60 g of alcohol per day (men) or 48 g of alcohol per day (women) for at least 5 days in the past 30 days prior to inclusion measured by the Time Line Follow Back Method (TLFB) method

Exclusion criteria

- Severe psychiatric disease, e.g. a diagnosis of schizophrenia, paranoid psychosis, bipolar disorder or mental retardation
- A history of delirium tremens or alcohol withdrawal seizures
- No serious withdrawal symptoms at inclusion (defined as a score higher than 9 on Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) at baseline examinations)
- Current or history of neurological disease including traumatic brain injury
- Current or history of diagnosis of type 1 or type 2 diabetes or plasma haemoglobin A1c (HbA1c) ≥48 moll/L at inclusion
- Females of child bearing potential who are pregnant, breast-feeding or have intention of becoming pregnant within the next 9 months, or are not using contraceptives (during the whole study period) considered as highly effective[29]
- Impaired hepatic function (liver transaminases >3 times upper normal limit)
- Impaired renal function (estimated Glomerular Filtration Rate (eGFR)) <50 mL/min)
- Impaired pancreatic function (any history of acute or chronic pancreatitis and/or amylase > 2 times upper limit)
- S-triglycerides > 10 moll/L
- History of medullary thyroid carcinoma (MTC) and/or family history with MTC and/or Multiple Endocrine neoplasia syndrome type 2 (MEN 2)[30]
- Cardiac problems defined as decompensated heart failure (New York Heart Association (NYHA) functional class III or IV), unstable angina pectoris and/or myocardial infarction within the last 12 months
- Uncontrolled hypertension (systolic blood pressure >180 mmHg, diastolic blood pressure >100 mmHg)
- Any prescribed use of anticoagulants within the last 12 months

- Concomitant pharmacotherapy against alcohol dependence i.e. disulfiram, naltrexone, acamprosate and nalmefene or treatment with any of these compounds within 1 month prior to inclusion
- Concomitant pharmacotherapy with dopamine active drugs, such as some types of Attention Deficit Hyperactivity Disorder (ADHD) medication (methylphenidate)
- Receiving any investigational drug within the last 3 months
- Use of weight-lowering pharmacotherapy within the preceding 3 month
- Any other active substance use defined as a Drug Use Disorders Identification Test (DUDIT)-score[31] > 6 (for men) >2 (for women) *and* fulfilling the criteria's for dependence of the substance according to the criteria of ICD-10 (except nicotine)
- BMI <18.5 kg/m^2
- Only for patients undergoing brain scans:
    - Contraindications for MR-scanning (magnetic implants, pacemaker, claustrophobia etc.)
    - Contraindications for SPECT-scanning (radiation exposure, excluding background radiation but including diagnostic x-rays and other medical exposures exceeding 10 mSv in the last 12 months, allergy towards iodine)
- Unable to speak and/or understand Danish
- Any condition that the investigator feels would interfere with trial participation

Time Line Follow Back Method

At week 0, 4, 12, 20 and 26, the examiner will – in close collaboration with the patient – fill out the TLFB-schedule for the last 30 days, based on the weekly collected alcohol diaries. The TLFB has been extensively tested and evaluated[32] and has, in addition, been demonstrated to have a high test-retest reliability in previous studies[33]. The information collected by the TLFB will be used to evaluate effects on *the primary endpoint*, i.e. number of heavy drinking days.

Blood analyses

At every examination, a variety of routine blood samples will be drawn. This is to monitor that the patients have no serious adverse reactions to the treatment compromising liver-, kidney-, haematological- or pancreatic function. Blood will also be drawn for two advanced tests, PEth and proteomics. PEth is the biomarker with the best correlation to self-reported alcohol consumption and it can reflect alcohol consumption during several weeks prior to sampling [34]. In our study we will be investigating the proteomic fingerprint as it is known that levels of humoral cytokines can be affected in alcohol related liver diseases and that GLP-1RAs have an additional impact on humoral cytokines[35].

Urine analyses

At baseline (week 0) and at the final examination (week 26) a urine sample will be collected. This is for a routine screening of albumin/creatinine-ratio and oxidative stress parameters. Oxidative stress, i.e. excessive reactive oxygen species (ROS), can cause cell-damaging effects through oxidative modification of macromolecules leading to their inappropriate functions. Such oxidative modification is related to cancers, aging, and neurodegenerative and cardiovascular diseases[36]. Studies in rats have shown that the GLP-1RA liraglutide may have a direct beneficial effect on oxidative stress and diabetic nephropathy[37].

Questionnaires

To assess potential psychopathology and drug use, as well as the level of alcohol use during the trial, a number of questionnaires will be administered at week 0 and at week 26 including: quality of life (Short Form Health Survey (SF-36)), psychopathology (Symptom Checklist (SCL-92)), depression symptoms (Major Depression Inventory (MDI)), alcohol consumption (AUDIT), craving (Penn Alcohol Craving Scale (PACS), smoking (Fagerström Test for Nicotine Dependence) and drug use (DUDIT).

Screen for Cognitive Impairment in Psychiatry-test

The Screen for Cognitive Impairment in Psychiatry (SCIP) is a brief (<20 min) and feasible neuropsychological instrument for screening for cognitive dysfunction in patients with psychotic and affective disorders[38] and in healthy controls[39]. It will be administered at week 0, 4 and 26 in three parallel equivalent forms to minimize learning effects.

Single-Photon Emission Computed Tomography

A subgroup of forty patients will have a SPECT-scan performed at baseline and after 26 weeks of treatment. We will use the SPECT brain scan with the dopamine transporter ligand 123I-2-b-carbomethoxy-3b-(4-iodophenyl)-N-(3-fluoropropyl) nortropane ([123I]-FP-CIT, DaTSCAN) administrated as a bolus injection. The [123I]-FP-CIT binding potential is used to calculate an estimate of DAT availability in regions of interest. As no human data are available on the effects of GLP-1RA in DAT availability, we also propose to investigate DAT availability in healthy, non-alcohol dependent subjects. The study on the healthy subjects is performed in order to investigate possible acute effects of exenatide on DAT availability in the human brain. Possible long-term effects of GLP-1R stimulation will be explored through scans at week 26 in the present study.

Functional Magnetic Resonance Imaging

The neuroanatomical underpinnings of the possible treatment effects will be investigated using fMRI at week 0 and 26. We will investigate brain activity during exposure to alcohol cues and during spatial working memory performance. Furthermore, we will evaluate the effects of exenatide versus placebo on functional connectivity in the brain during resting state, on structural connectivity and brain morphology. A subgroup of fifty patients will have an fMRI-scan performed at baseline and after 26 weeks of treatment. The patients will undergo two different tasks presented in block paradigms to maximize sensitivity for blood-oxygen-level dependent (BOLD) signal change. In the first task, the patients will be shown a series of alcohol related and neutral pictures. Following each block, the patients rate the intensity of their alcohol craving on a visual analogue scale[9]. In the second task the spatial working memory (SWM) will be assessed using an N-back version design[40]. To obtain comparable data from healthy controls in the alcohol and spatial working memory paradigms (fMRI), we will scan 25 healthy participants. The participants will have no history of alcohol dependence and will be matched to the patients with respect to gender and age.

Sample size calculation and randomization

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[41], where the reduction in the percentage of total number of heavy drinking days was 60.34 % in the intervention group and 32.73 % in the control group, with an alpha of 5 %, and a power of 90%, and with an estimated SD of 34.5, 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) are needed.

The patients will be randomized into two groups with 57 patients in each group using the randomization module in Research Electronic Data Capture (REDCap). The randomization will be stratified in terms of age, sex and baseline alcohol consumption (i.e. number of heavy drinking days measured by TLFB). Patients, investigators, other care givers performing assessments and persons performing data analysis will remain blinded from the time of randomization until time of database unlock. In order to maintain the blinding of the patients, an un-blinded nurse will perform the randomization and prepare the injections. If a patient develops an adverse reaction that requires knowledge of the treatment the randomization will be broken for only that particular patient.

Statistical analysis

Before dividing participants into two groups, the statistical analysis plan will be uploaded at clinicaltrials.gov. Analyses will be made by use of R software[42], with alpha set at 0.05 and two-sided testing. 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 (exenatide or placebo). Missing data will be imputed using multiple imputations, and a sensitivity analysis will be undertaken to evaluate and compare imputation results to complete case analyses. Multiple linear regression and logistic regression analyses will be used for the analyses, where we will control for possible confounders, e.g. baseline alcohol consumption, social status, age etc. in addition to the treatment.

Endpoints

The primary endpoint is percent reduction in total number of heavy drinking days, defined as days with an excess intake of 60/48 grams of alcohol per day (men and women, respectively) the previous 30 days from baseline to follow-up after 26 weeks of treatment, measured by TFLB method.

The secondary endpoints include changes in total alcohol consumption (g/30 days measured by TLFB), changes in number of days without alcohol consumption and PACS score, change in AUDIT score, change in DUDIT score, change in cognitive performance on the SCIP-test, change in the liver parameters gamma-glutamyltransferase (GGT), alanine aminotransferase (ALAT) and PEth. Other parameters will be mean cell volume (MCV), changes in body weight, blood pressure, pulse, overall glycaemic control parameters (HbA1c), kidney function (p-creatinine, eGFR and urine albumin/creatinine ratio) and measures of health (SF-36 and SCL-92).

In addition to these clinical outcome parameters, we will explore the possible neuromolecular effects by measuring striatal DAT availability before and after administration of exenatide by use of SPECT. The possible neuroanatomical underpinnings of exenatide will be investigated by use of fMRI. Both examinations will be performed in two subgroups of patients treated with either exenatide or placebo. To have comparable standard data in this fMRI alcohol related paradigm, we will include 25 healthy participants with no record of alcohol dependence.

Ethical considerations

The study is approved by The Regional Committee on Biomedical Research Ethics (journal number H-17003043), The Danish Data Protection Agency (protocol number RHP-2017-029) and the Danish Medical Agency (EudraCT 2016-003343-11). On ClinicalTrials.gov it can be identified by the ID NCT03232112.

Discussion

Data from animal studies suggest that the inhibitory effects of the GLP-1RA exendin-4 reduce alcohol consumption in rodents and this effect is likely mediated by stimulation of the dopamine transporters[43]. So far no human studies have been performed and the present trial therefore serves to investigate the effects of the GLP-1RA exenatide on alcohol consumption in patients with alcohol dependence as well as the associated neurobiological mechanisms. This trial is the first RCT to investigate the effects of GLP-1R stimulation on alcohol consumption in patients diagnosed with alcohol dependence.

Limitations

The measurement of the primary endpoint of the study, i.e. change in heavy drinking days, is self-reported and retrospective, and might therefore have poor reliability. In the present study we use the TLFB method which has been extensively tested and evaluated[32]. Self-reported measurements can be influenced by several factors including social factors characteristics in the respondent group[44]. For example, it is known that patients with alcohol dependence tend to describe themselves more negatively, i.e. having more heavy drinking days etc., than suggested by data from more objective sources, e.g. blood samples[32]. However, when patients have alcohol in the blood, the opposite is seen, i.e. an underestimation of the alcohol intake[32]. Thus, to limit the possible bias from different factors, the TLFB will be filled out in close cooperation with the patient in a standardized setting. In addition, the patients will do a breath alcohol test prior to all examinations.

Another limitation of the study is that the treatment is not evaluated long-term. We also considered adding a third arm comparing exenatide to one of the established add-on treatments (all pharmacological treatments is considered as an add-on to CBT). However, adding a third arm would have increased the complexity and cost of the trial considerably. A weakness of the present study is the lack of blinded placebo pens making weekly injections of exenatide by the study nurse necessary, which increases the risk of selection bias, as the design requires a very compliant patient, i.e. patients having less resources might not participate. Additionally, some patients might choose not to participate because of needle phobia.

Strengths

A significant advantage of the present study is the extensive use of unbiased, biological measurements, i.e. biomarkers in blood- and urine and brain scans. A systematic review of the biomarker PEth thus showed a significant statistical difference when comparing heavy drinkers (i.e. >60 grams of alcohol per day) from persons consuming less[45], making it very useful in the present study as we will be able to assess the correlation between the self-reported alcohol intake and PEth. Another advantage is the use of the brain imaging techniques SPECT and fMRI. The brain scans will allow the investigation of the possible neuroanatomical underpinnings of the treatment. A definite strength of the study is the long treatment period, i.e. 26 weeks, when comparing to similar studies with study durations of typically 8-12 weeks[46,47]. This relatively long treatment period will allow a better understanding of the true effects of exenatide as it corresponds to a more realistic setting with an ongoing risk of relapse persisting way longer than just a few months[4]. Also, the design, i.e. double-blinded, randomized, placebo-controlled, is an advantage as it reduces experimental bias, ensures balance in the two treatment groups and gives a direct estimate of the possible effect of exenatide. In addition, the present injection set up allows us to verify, that the injections have been administered to the patients.

Perspectives

If GLP-1R stimulation proves efficacious in the treatment of alcohol dependence, it can be implemented in future treatment relatively easy as exenatide is already used in the clinic and the injections are designed for self-distribution. Further, per oral GLP-1RAs may be on the marked within a few years, which would possible increase compliance even more. In addition, assessment of the neuronal underpinnings of the potential treatment effects will increase insight into neurobiological targets for future treatments.

Trial status

Patient enrolment started in July 2017 and is ongoing until 114 patients have been randomized and received first injection.

Contributor ship statement

Authors Anders Fink-Jensen and Tina Vilsbøll made the first draft of the study protocol and all authors have made substantial contributions to the study design. Kerstin K. Antonsen, Mette K. Klausen, Claus Ekstrøm and Anders Fink-Jensen undertook the statistical power calculations. Kamilla W. Miskowiak, Patrick M. Fisher, Mette K. Klausen, Gerda K. Thomsen and Gitte M. Knudsen undertook the final design of the fMRI experiment. Kerstin K. Antonsen, Gerda K. Thomsen and Gitte M. Knudsen Knudsen undertook the final design of the SPECT experiment analysis, and author Kerstin K. Antonsen wrote the first draft of the manuscript based on the study protocol. All authors (Kerstin K. Antonsen, Mette K. Klausen, Amanda S. Brunchmann, Nina le Dous, Mathias E. Jensen, Kamilla W. Miskowiak, Patrick M. Fisher, Gerda K. Thomsen, Henrik Rindom, Thomas P. Fahmy, Sabine Vollstädt-Klein, Helene Benveniste, Nora Volkow, Ulrik Becker, Claus Ekstrøm, Gitte M. Knudsen, Tina Vilsbøll and Anders Fink-Jensen) contributed with critical revision of the manuscript for important intellectual content and have approved the final manuscript.
Thus, all authors have been revising the study protocol and article critically, given their final approval of the version to be published and agreed to be accountable for all aspects of the content of the article.

Competing interests

Tina Vilsbøll has received lecture fees from Amgen, Astra Zeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Sanofi, and Zealand Pharma, and is a member of the Advisory Boards of Astra Zeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck, Sharp & Dohme, Novo Nordisk and Sanofi.
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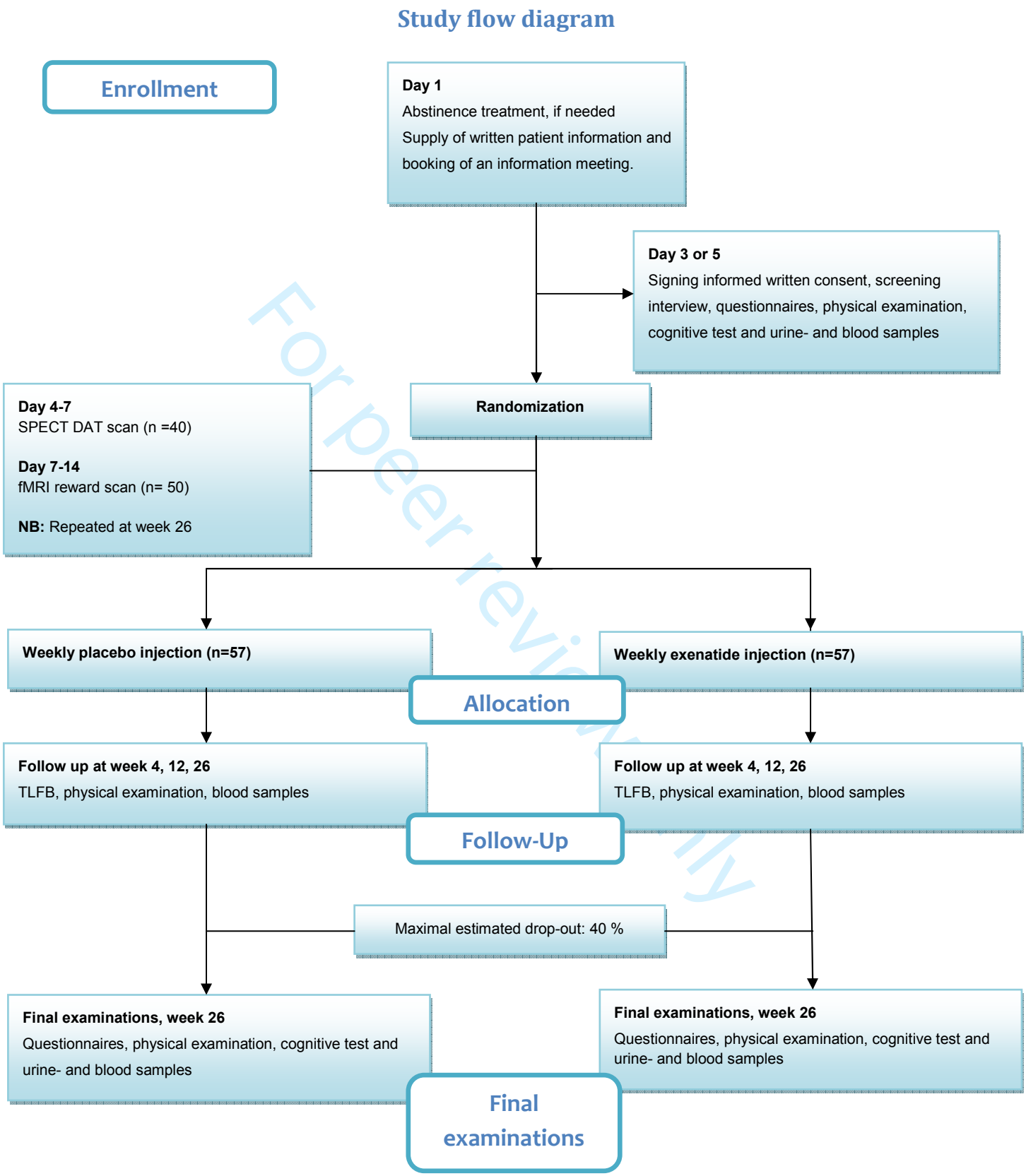

Figure 1. Study flow diagram. SPECT, Single-photon emission computed tomography; fMRI, functional Magnetic Resonance Imaging; TLFB, Time Line Follow Back.

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Does glucagon-like peptide-1 (GLP-1) receptor agonist stimulation reduce alcohol intake in patients with alcohol dependence? Study protocol of a randomized, double-blinded, placebo-controlled clinical trial

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Abstract

Introduction: Alcohol dependence is a major public health problem. It is under-diagnosed and undertreated. Even when treated, more than 2/3 of patients in abstinence-oriented treatment will relapse within the first year. Thus, there is an urgent need for efficacious medical treatment of alcohol dependence. Glucagon-like peptide-1 (GLP-1) receptor stimulation has proven to reduce alcohol consumption in preclinical experiments. However, the effect of GLP-1 receptor agonists in humans has to our knowledge, not yet been investigated. **Methods and analysis**: *Design, participants and intervention*: The effect of the once-weekly GLP-1-receptor-agonist exenatide will be investigated in a double-blinded, placebo-controlled, randomized clinical trial. One hundred and fourteen outpatients will be recruited and randomized to treatment with either placebo or exenatide once-weekly for 26 weeks as a supplement to cognitive behavioural therapy. *The primary endpoint* is reduction in number of 'heavy drinking days'. *The secondary endpoints* include changes in total alcohol consumption, days without consumption, changes in brain activity and function, smoking status, cognition, measures of quality of life and changes in phosphatidylethanol (PEth) as a biomarker of alcohol consumption from baseline to follow-up at week 26. *Status*: Currently recruiting patients. **Ethics and dissemination**: Ethical approval has been obtained. Before screening, all patients will be provided oral and written information about the trial. The study results will be disseminated by peer-review publications and conference presentations and has the potential to reveal a completely new medical treatment of alcohol dependence.

Strengths and limitations of this study

- The study design, i.e. a double-blinded, randomized, placebo-controlled clinical trial, is a strength as it is designed to evaluate the effects of the GLP-1 receptor agonist exenatide on alcohol consumption in patients with alcohol dependence
- The study duration is 26 weeks which is longer than most previous studies investigating medical treatment of alcohol dependence
- The biological basis for any demonstrated effect is investigated with brain imaging techniques and the biomarker phosphatidylethanol (PEth)
- A possible limitation is that the alcohol intake is self-reported which potentially could affect accuracy
- Another limitation is that the study has no third treatment arm for comparing exenatide to one of the known compounds used in the clinic against alcohol use disorder, e.g. disulfiram, acamprosate or naltrexone.

Introduction

Alcohol dependence is a major global public health problem across the world[1,2]. It is an under-diagnosed and undertreated[3] condition and more than 2/3 of patients in abstinence-oriented treatment will relapse within the first year[4]. In Denmark, approximately 20% of the population is consuming more alcohol than recommended by the Danish National Board of Health[5]. Further, 8.5% of the Danish adult population (16 years or older) has a 'risky', i.e. potentially harmful, alcohol consumption, defined as more than 14 and 21 units of alcohol (one unit defined as 12 grams of pure alcohol) for women and men per week, respectively[6]. Three percent fulfils the criteria for alcohol dependence[7].

Psychological treatment

One of the best documented treatments of alcohol dependence is cognitive behavioural therapy (CBT)[8]. The underlying neuroanatomical basis of alcohol addiction and treatment effects of CBT are not yet established, although functional Magnetic Resonance Imaging (fMRI) studies have begun to elucidate the neural underpinnings of alcohol dependence[9]. Alcohol dependent patients have been found to display increased dorsal anterior cingulate cortex (dACC) activation during spatial working memory, perhaps reflecting decreased prefrontal efficiency because of distracting alcohol related thoughts[10]. Interestingly, it was also recently demonstrated that alcohol dependent patients also display increased neural activation to alcohol associated cues in mesocortico-limbic networks; which is normalized with psychological therapy[9].

Pharmacological treatment

Pharmacological treatment of alcohol dependence is considered an important supplement to psychological therapy[11]. *Disulfiram*, a substance that blocks alcohol-metabolizing enzymes resulting in increased acetaldehyde concentrations, was introduced in Denmark in 1948[12]. Newer pharmacological agents such as *Acamprosate* a gamma-amino-butyric-acid (GABA) receptor agonist and the glutamate N-Methyl-D-aspartate (NMDA) receptor antagonist; and *naltrexone*, a mu and kappa opioid receptor antagonist are now used as alternate treatments for alcohol dependence. However, these compounds have not gained widespread dissemination, probably because the effect of the substances is modest, with a less than 10% increase in abstinent rate compared to placebo[13]. The antiepileptic compound *topiramate* has shown promising results in clinical trials[14] and another pharmacological agent, *nalmefene*, with a mechanism of action somewhat similar to naltrexone, has very recently been approved by the European Medicines Agency

(EMA) as a medication for reducing alcohol consumption[15]. Clearly, given the moderate success rates of CBT[8] and the synergistic effects of adding pharmacological treatment – as described above – are quite limited, there is an urgent need for new and more efficient treatment modalities of alcohol dependence.

Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists (GLP-1RA)

GLP-1 based therapy for the treatment of type 2 diabetes was introduced in 2006[16]. 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[17]. GLP-1 conveys an insulinotropic effect through GLP-1 receptors (GLP-1R) on the beta cells of the pancreas and inhibits the secretion of glucagon from the alpha cells of the pancreas, which lower the blood glucose level[18]. Naturally occurring GLP-1 is rapidly degraded within minutes by the enzyme, dipeptidyl peptidase 4 (DPP-4)[17]. 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. Exenatide binds to the GLP-1R with high affinity[19] and acts as a receptor agonist, thus referred to as a GLP-1RA.

GLP-1RA: A potential new treatment for alcohol use disorder?

GLP-1RA has a well-established effect on the food reward system which seems to be driven by two key mesolimbic brain regions, the ventral tegmental area (VTA) and nucleus accumbens (NAc)[20]. These regions are not only involved in the rewarding properties of food but also to drugs of abuse, including alcohol[21,22,23]. Interestingly, GLP-1 receptors (GLP-1R) are expressed in these brain reward regions (VTA and NAc), which are innervated by hindbrain GLP-1 neurons[21]. A link between alcohol intake and GLP-1 has been demonstrated in studies and is considered to play a central role in development of addiction to stimulant drugs (i.e. cocaine, amphetamine, alcohol)[24]. The findings are consistent with the hypothesis, that systemic administration of GLP-1RA can influence the mesolimbic dopamine system and reward-seeking behaviours associated with alcohol dependence[25]. Although the precise mechanism of action has not been elucidated *in vivo*, we recently reported that *in vitro*, exendin-4 induces an upregulation of the dopamine transporter (DAT) function[26].

Given this collective evidence, we aim to investigate whether the beneficial effect of the GLP-1 receptor agonist, exenatide, on alcohol consumption in preclinical studies, can be translated to patients with known alcohol use disorder[27].

Hypothesis

- Exenatide treatment will decrease alcohol consumption, measured as total number of heavy drinking days, in alcohol dependent patients.
- Exenatide will induce upregulation of the striatal dopamine transporter availability, in alcohol dependent patients.
- Exenatide will modulate neural responses in reward processing regions including nucleus accumbens.

To test these hypothesis, we have designed a 26-week, clinical trial including 114 patients with known alcohol dependence. To explore the underlying neuromolecular mechanism(s) of the potential positive effect of exenatide vs. placebo on alcohol consumption, we will obtain Single-Photon Emission Computed Tomography (SPECT) neuroimaging of DAT at week 0 and 26 in a subgroup of the patients. Further, the functional brain network modulated by the possible treatment effects will be investigated using fMRI at week 0 and 26.

Methods and analysis

Study design

The present study is a 26-week, double-blinded, randomized, placebo-controlled clinical trial, designed to evaluate the effects of exenatide vs. placebo in 114 patients, diagnosed with alcohol dependence according to the International Classification of Diseases, tenth edition (ICD-10) and with an alcohol use disorder according to the The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria. The patients will be recruited from the Novavì outpatient clinics in Copenhagen, Denmark. To be eligible for participation, the patients will first undergo screening according to the inclusion and exclusion criteria. When consented and included, the patient will meet to get his/her weekly injection by the un-blinded nurse. The nurse will collect a weekly alcohol diary and hand out a new one, for the following week.

Participants and screening

The patients will be recruited from outpatient units, specifically, the Novavì outpatient clinics in suburbs of Copenhagen, Denmark. All patients will receive psychosocial alcohol treatment based on psycho educative elements, motivational interviewing and CBT. Skilled staff members will be administering the psychosocial treatment in the clinic. The recruitment procedure starts as a pre-screening when the patients contact the Novavì outpatient clinics, which are open outpatient clinics. All potentially eligible patients will be fully informed, verbally as well as in writing, of their rights and responsibilities while participating in the trial. Screening examinations will only be performed after the patient has agreed to participate and has signed the informed consent form.

At the time of screening the patients will undergo a series of examinations to assure that all in- and exclusion criteria are met. The patients will be asked general information about psychosocial factors, i.e., education level, employment- and marital status. In addition, somatic symptoms and baseline medications will be registered. Blood samples and a urine tests will be collected for acute analysis according to the exclusion criteria. Furthermore, blood- and urine samples will be saved for an investigational biobank that will allow more advanced analyses, e.g. phosphatidylethanol (PEth). The most important tests and examinations are described in details in later sections. See also the figure 1 for a complete schedule of events. We regularly evaluate the inclusion frequency, and we have the option of including more trial centres to assure recruitment.

Intervention

The pharmacological intervention will be given as an add-on to the standardized psychosocial alcohol treatment paradigm. Exenatide is delivered from *Region Hovedstadens Apotek* as a powder with solvent for prolonged release injection (once-weekly). Each single-dose, dual-chamber pen contains 0.65 ml of diluent and 2 mg of exenatide, which are isolated until mixed by the nurse administering the drug. The placebo will be supplied as pre-filled saline syringes (0.9% saline), by *Region Hovedstadens Apotek* and will be administered in the same way and volume as exenatide. The un-blinded nurse, with no involvement in the psychosocial treatment, will administer the drug injections, and the patients will be blindfolded while receiving the once-weekly injections. To promote participant retention, the nurse will contact the patient if he or she does not show up for the weekly injection.

Inclusion criteria

- Informed oral and written consent

- Diagnosed with alcohol dependence according to the criteria of ICD-10, World Health Organization and DSM-5 (for the equivalent diagnosis of alcohol use disorder)
- Alcohol use disorder identification test (AUDIT) score >15[28]
- Age 18 - 70 years (both included)
- Heavy alcohol drinking defined as having alcohol consumption over 60 g of alcohol per day (men) or 48 g of alcohol per day (women) for at least 5 days in the past 30 days prior to inclusion measured by the Time Line Follow Back Method (TLFB) method

Exclusion criteria

- Severe psychiatric disease, e.g. a diagnosis of schizophrenia, paranoid psychosis, bipolar disorder or mental retardation
- A history of delirium tremens or alcohol withdrawal seizures
- No serious withdrawal symptoms at inclusion (defined as a score higher than 9 on Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) at baseline examinations)
- Current or history of neurological disease including traumatic brain injury
- Current or history of diagnosis of type 1 or type 2 diabetes or plasma haemoglobin A1c (HbA1c) ≥48 moll/L at inclusion
- Females of child bearing potential who are pregnant, breast-feeding or have intention of becoming pregnant within the next 9 months, or are not using contraceptives (during the whole study period) considered as highly effective[29]
- Impaired hepatic function (liver transaminases >3 times upper normal limit)
- Impaired renal function (estimated Glomerular Filtration Rate (eGFR)) <50 mL/min)
- Impaired pancreatic function (any history of acute or chronic pancreatitis and/or amylase > 2 times upper limit)
- S-triglycerides > 10 moll/L
- History of medullary thyroid carcinoma (MTC) and/or family history with MTC and/or Multiple Endocrine neoplasia syndrome type 2 (MEN 2)[30]
- Cardiac problems defined as decompensated heart failure (New York Heart Association (NYHA) functional class III or IV), unstable angina pectoris and/or myocardial infarction within the last 12 months
- Uncontrolled hypertension (systolic blood pressure >180 mmHg, diastolic blood pressure >110 mmHg)
- Concomitant pharmacotherapy against alcohol dependence i.e. disulfiram, naltrexone, acamprosate and nalmefene or treatment with any of these compounds within 1 month prior to inclusion
- Concomitant pharmacotherapy with dopamine active drugs, such as some types of Attention Deficit Hyperactivity Disorder (ADHD) medication (methylphenidate)
- Receiving any investigational drug within the last 3 months
- Use of weight-lowering pharmacotherapy within the preceding 3 month
- Any other active substance use defined as a Drug Use Disorders Identification Test (DUDIT)-score[31] > 6 (for men) >2 (for women) *and* fulfilling the criteria's for dependence of the substance according to the criteria of ICD-10 (except nicotine)
- BMI <18.5 kg/m^2
- Only for patients undergoing brain scans:

  - Contraindications for MR-scanning (magnetic implants, pacemaker, claustrophobia etc.)
  - Contraindications for SPECT-scanning (radiation exposure, excluding background radiation but including diagnostic x-rays and other medical exposures exceeding 10 mSv in the last 12 months, allergy towards iodine)
- Unable to speak and/or understand Danish
- Any condition that the investigator feels would interfere with trial participation

Withdrawal criteria

Patients are free to withdraw from the trial at any time without providing a reason therefore and without impact on further treatment at *Novavì ambulatorierne*. The reason for withdrawal may be withdrawal of consent, treatment failure, adverse event, pregnancy discovered during the trial, or profound increase in alcohol consumption. Failure to comply with clinical trial medication, i.e. if the patient misses more than three consecutive injections or more than five injections in total leads to exclusion.

Time Line Follow Back Method

At week 0, 4, 12, 20 and 26, the examiner will – in close collaboration with the patient – fill out the TLFB-schedule for the last 30 days, based on the weekly collected alcohol diaries. The TLFB has been extensively tested and evaluated[32] and has, in addition, been demonstrated to have a high test-retest reliability in previous studies[33]. The information collected by the TLFB will be used to evaluate effects on *the primary endpoint*, i.e. number of heavy drinking days.

Blood analyses

At every examination, a variety of routine blood samples will be drawn. This is to monitor that the patients have no serious adverse reactions to the treatment compromising liver-, kidney-, or pancreatic function. HbA1c will only be analysed at week 0 and week 26. At week 0 and week 26, blood will also be drawn for two advanced tests, proteomics and bone markers. At every examination blood will be drawn for the advanced test PEth which is the biomarker with the best correlation to self-reported alcohol consumption and it can reflect alcohol consumption during several weeks prior to sampling 34. In the present study we will be investigating the proteomic fingerprint as it is known that levels of humoral cytokines can be affected in alcohol related liver diseases and that GLP-1RAs have an additional impact on humoral cytokines35. We will also measure plasma levels of the bone markers collagen type 1 C-telopeptide (CTX) and procollagen type 1 N-terminal propeptide (P1NP), as former studies show that another incretin hormone (GIP) reduces bone resorption36.

Urine analyses

At baseline (week 0) and at the final examination (week 26) a urine sample will be collected. This is for a routine screening of albumin/creatinine-ratio and oxidative stress parameters. Oxidative stress, i.e. excessive reactive oxygen species (ROS), can cause cell-damaging effects through oxidative modification of macromolecules leading to their inappropriate functions. Such oxidative modification is related to cancers, aging, and neurodegenerative and cardiovascular diseases[37]. Studies in rats have shown that the GLP-1RA liraglutide may have a direct beneficial effect on oxidative stress and diabetic nephropathy[38].

Questionnaires

To assess potential psychopathology and drug use, as well as the level of alcohol use during the trial, a number of questionnaires will be administered at week 0 and at week 26 including: quality of life (Short

Form Health Survey (SF-36)), psychopathology (Symptom Checklist (SCL-92)), depression symptoms (Major Depression Inventory (MDI)), alcohol consumption (AUDIT), craving (Penn Alcohol Craving Scale (PACS), smoking (Fagerström Test for Nicotine Dependence) and drug use (DUDIT).

Screen for Cognitive Impairment in Psychiatry-test

The Screen for Cognitive Impairment in Psychiatry (SCIP) is a brief (<20 min) and feasible neuropsychological instrument for screening for cognitive dysfunction in patients with psychotic and affective disorders[39] and in healthy controls[40]. It will be administered at week 0, 4 and 26 in three parallel equivalent forms to minimize learning effects.

Single-Photon Emission Computed Tomography

A subgroup of forty patients will have a SPECT-scan performed at baseline and after 26 weeks of treatment. We will use the SPECT brain scan with the dopamine transporter ligand 123I-2-b-carbomethoxy-3b-(4-iodophenyl)-N-(3-fluoropropyl) nortropane ([123I]-FP-CIT, DaTSCAN) administrated as a bolus injection. The [123I]-FP-CIT binding potential is used to calculate an estimate of DAT availability in regions of interest. As no human data are available on the effects of GLP-1RA in DAT availability, we also propose to investigate DAT availability in healthy, non-alcohol dependent subjects. The study on the healthy subjects is performed in order to investigate possible acute effects of exenatide on DAT availability in the human brain. Possible long-term effects of GLP-1R stimulation will be explored through scans at week 26 in the present study.

Functional Magnetic Resonance Imaging

The neuroanatomical underpinnings of the possible treatment effects will be investigated using fMRI at week 0 and 26. We will investigate brain activity during exposure to alcohol cues and during spatial working memory performance. Furthermore, we will evaluate the effects of exenatide versus placebo on functional connectivity in the brain during resting state, on structural connectivity and brain morphology. A subgroup of fifty patients will have an fMRI-scan performed at baseline and after 26 weeks of treatment. The patients will undergo two different tasks presented in block paradigms to maximize sensitivity for blood-oxygen-level dependent (BOLD) signal change. In the first task, the patients will be shown a series of alcohol related and neutral pictures. Following each block, the patients rate the intensity of their alcohol craving on a visual analogue scale[9]. In the second task the spatial working memory (SWM) will be assessed using an N-back version design[41]. To obtain comparable data from healthy controls in the alcohol and spatial working memory paradigms (fMRI), we will scan 25 healthy participants. The participants will have no history of alcohol dependence and will be matched to the patients with respect to gender and age.

Sample size calculation and randomization

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[42], where the reduction in the percentage of total number of heavy drinking days was 60.34 % in the intervention group and 32.73 % in the control group, with an alpha of 5 %, and a power of 90%, and with an estimated SD of 34.5, 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) are needed.

The patients will be randomized into two groups with 57 patients in each group using the randomization module in Research Electronic Data Capture (REDCap). The randomization will be stratified in terms of age (two levels), sex (two levels) and baseline alcohol consumption (i.e. number of heavy drinking days meas-

ured by TLFB) (four levels). The block sizes will be randomised between 2 and 4. The random allocation sequence will be generated by an extern statistician by use of the R statistical package blockrand and uploaded in REDCap in accordance with REDCap's user guide and reference manual[43].
Patients, investigators, other care givers performing assessments and persons performing data analysis will remain blinded from the time of randomization until time of database unlock. In order to maintain the blinding of the patients, an un-blinded nurse will perform the randomization and prepare the injections. If a patient develops, an adverse reaction that requires knowledge of the treatment the randomization will be broken for only that particular patient.

Patient and public involvement

No patients were involved in development of the research question or in designing the study, and the burden of the intervention is not assessed by patients themselves.
When signing the informed consent, patients are encouraged to fill in their e-mail address, so they can receive the results of the study.

Statistical analysis

Before dividing participants into two groups, the statistical analysis plan will be uploaded at clinicaltrials.gov. Analyses will be made by use of R software[44], with alpha set at 0.05 and two-sided testing. 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 (exenatide or placebo). Missing data will be imputed using multiple imputations, and a sensitivity analysis will be undertaken to evaluate and compare imputation results to complete case analyses. Multiple linear regression and logistic regression analyses will be used for the analyses, where we will control for possible confounders, e.g. baseline alcohol consumption, social status, age etc. in addition to the treatment.

Endpoints

The primary endpoint is percent reduction in total number of heavy drinking days, defined as days with an excess intake of 60/48 grams of alcohol per day (men and women, respectively) the previous 30 days from baseline to follow-up after 26 weeks of treatment, measured by TFLB method.

The secondary endpoints include changes in total alcohol consumption (g/30 days measured by TLFB), changes in number of days without alcohol consumption and PACS score, change in AUDIT score, change in DUDIT score, change in cognitive performance on the SCIP-test, change in the liver parameters gamma-glutamyltransferase (GGT), alanine aminotransferase (ALAT) and PEth. Other parameters will be mean cell volume (MCV), changes in body weight, blood pressure, pulse, overall glycaemic control parameters (HbA1c), kidney function (p-creatinine, eGFR and urine albumin/creatinine ratio) and measures of health (SF-36 and SCL-92).

In addition to these clinical outcome parameters, we will explore the possible neuromolecular effects by measuring striatal DAT availability before and after administration of exenatide by use of SPECT. The possible neuroanatomical underpinnings of exenatide will be investigated by use of fMRI. Both examinations will be performed in two subgroups of patients treated with either exenatide or placebo. To have comparable standard data in this fMRI alcohol related paradigm, we will include 25 healthy participants with no record of alcohol dependence.

The CONSORT guidelines will be followed when final study data are reported.

Ethical considerations

The study is approved by The Regional Committee on Biomedical Research Ethics (journal number H-17003043), The Danish Data Protection Agency (protocol number RHP-2017-029) and the Danish Medical Agency (EudraCT 2016-003343-11). On ClinicalTrials.gov it can be identified by the ID NCT03232112. Please see Appendix A for further details. The protocol has version control and dates as identifiers. Any amendments have to be approved by the above-mentioned authorities before implementation.

Discussion

Data from animal studies suggest that the inhibitory effects of the GLP-1RA exendin-4 reduce alcohol consumption in rodents and this effect is likely mediated by stimulation of the dopamine transporters26. So far no human studies have been performed and the present trial therefore serves to investigate the effects of the GLP-1RA exenatide on alcohol consumption in patients with alcohol dependence as well as the associated neurobiological mechanisms. This trial is the first RCT to investigate the effects of GLP-1R stimulation on alcohol consumption in patients diagnosed with alcohol dependence.

Limitations

The measurement of the primary endpoint of the study, i.e. change in heavy drinking days, is self-reported and retrospective, and might therefore have poor reliability. In the present study we use the TLFB method which has been extensively tested and evaluated[32]. Self-reported measurements can be influenced by several factors including social factors characteristics in the respondent group[45]. For example, it is known that patients with alcohol dependence tend to describe themselves more negatively, i.e. having more heavy drinking days etc., than suggested by data from more objective sources, e.g. blood samples[32]. However, when patients have alcohol in the blood, the opposite is seen, i.e. an underestimation of the alcohol intake[32]. Thus, to limit the possible bias from different factors, the TLFB will be filled out in close cooperation with the patient in a standardized setting. In addition, the patients will do a breath alcohol test prior to all examinations.

Another limitation of the study is that the treatment is not evaluated long-term. We also considered adding a third arm comparing exenatide to one of the established add-on treatments (all pharmacological treatments is considered as an add-on to CBT). However, adding a third arm would have increased the complexity and cost of the trial considerably. A weakness of the present study is the lack of blinded placebo pens making weekly injections of exenatide by the study nurse necessary, which increases the risk of selection bias, as the design requires a very compliant patient, i.e. patients having less resources might not participate. Additionally, some patients might choose not to participate because of needle phobia.

Strengths

A significant advantage of the present study is the extensive use of unbiased, biological measurements, i.e. biomarkers in blood- and urine and brain scans. A systematic review of the biomarker PEth thus showed a significant statistical difference when comparing heavy drinkers (i.e. >60 grams of alcohol per day) from persons consuming less[46], making it very useful in the present study as we will be able to assess the correlation between the self-reported alcohol intake and PEth. Another advantage is the use of the brain imaging techniques SPECT and fMRI. The brain scans will allow the investigation of the possible neuroanatomical

underpinnings of the treatment. A definite strength of the study is the long treatment period, i.e. 26 weeks, when comparing to similar studies with study durations of typically 8-12 weeks[47,48]. This relatively long treatment period will allow a better understanding of the true effects of exenatide as it corresponds to a more realistic setting with an ongoing risk of relapse persisting way longer than just a few months[4]. Also, the design, i.e. double-blinded, randomized, placebo-controlled, is an advantage as it reduces experimental bias, ensures balance in the two treatment groups and gives a direct estimate of the possible effect of exenatide. In addition, the present injection set up allows us to verify, that the injections have been administered to the patients.

Perspectives

If GLP-1R stimulation proves efficacious in the treatment of alcohol dependence, it can be implemented in future treatment relatively easy as exenatide is already used in the clinic and the injections are designed for self-distribution. Further, per oral GLP-1RAs may be on the market within a few years, which would possible increase compliance even more. In addition, assessment of the neuronal underpinnings of the potential treatment effects will increase insight into neurobiological targets for future treatments.

Trial status

Patient enrolment started in August 2017 and is ongoing until 114 patients have been randomized and received first injection.

Funding statement

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Competing interest statement

Tina Vilsbøll has received lecture fees from Amgen, Astra Zeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Sanofi, and Zealand Pharma, and is a member of the Advisory Boards of Astra Zeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck, Sharp & Dohme, Novo Nordisk and Sanofi. Anders Fink-Jensen has received an unrestricted grant from Novo Nordisk A/S for another project.

Contributorship statement

According to the definition given by the International Committee of Medical Journal Editors (ICMJE), all the authors qualify for authorship. AFJ and TV made the first draft of the study protocol. KKA, MKK, ASB, NLD, MEJ, KWM, HR, TPF, HB, NV, GMK and UB have made substantial contributions to the study design. KKA, MKK, CE and AFJ undertook the statistical power calculations. KWM, PMF, MKK, GKT and GMK undertook the final design of the fMRI experiment. SVK has designed the ALCUE fMRI-paradigm. KKA, GKT and GMK undertook the final design of the SPECT experiment. KKA wrote the first draft of the manuscript based on the study protocol. All authors contributed with critical revision of the manuscript for important intellectual content and have approved the final manuscript.

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Figure Legends

Figure 1 Study flow diagram. SPECT, Single-photon emission computed tomography; fMRI, functional Magnetic Resonance Imaging; TLFB, Time Line Follow Back.

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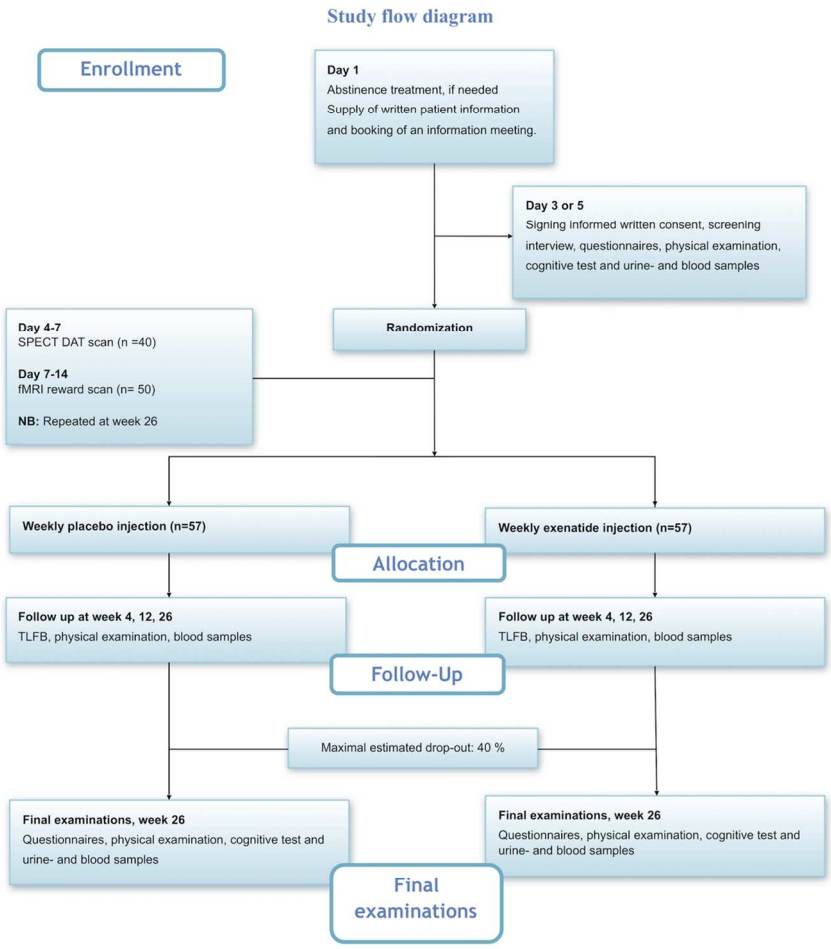

Figure 1: Study flow diagram. SPECT, Single-photon emission computed tomography; fMRI, functional Magnetic Resonance Imaging; TLFB, Time Line Follow Back.

109x141mm (300 x 300 DPI)

Appendix A - World Health Organization Trial Registration Data Set

DATA CATEGORY	INFORMATION
Primary registry and trial identifying number	ClinicalTrials.gov NCT03232112
Date of registration in primary registry	20.07.17
Secondary identifying numbers	The Regional Committee on Biomedical Research Ethics (journal number H-17003043), The Danish Data Protection Agency (protocol number RHP-2017-029) and the Danish Medical Agency (EudraCT 2016-003343-11)
Source(s) of monetary or material support	Region Hovedstadens Forskningsfond, Region Hovedstadens Psykiatri and Fonden Novavì. The manufacturer of Bydureon®, AstraZeneca A/S, has no financial interest or involvement in this project.
Primary sponsor	Fonden Novavì
Secondary sponsor(s)	Region Hovedstadens Forskningsfond, Region Hovedstadens Psykiatri
Contact for public queries	Mette Kruse Klausen MD, Psychiatric Centre Copenhagen, Rigshospitalet, Copenhagen University Hospital, Denmark
Contact for scientific queries	Anders Fink-Jensen MD DMSc, Department O (Rigshospitalet), Psychiatric Centre Copenhagen, Copenhagen University Hospital, Denmark
Public title	Does glucagon-like peptide-1 (GLP-1) receptor agonist stimulation reduce alcohol intake in patients with alcohol dependence? Study protocol of a randomized, double-blinded, placebo-controlled clinical trial
Scientific title	Does glucagon-like peptide-1 (GLP-1) receptor agonist stimulation reduce alcohol intake in patients with alcohol dependence? Study protocol of a randomized, double-blinded, placebo-controlled clinical trial
Countries of recruitment	Denmark
Health condition(s) or problem(s) studied	Addiction

Appendix A - World Health Organization Trial Registration Data Set

Intervention(s)	2 mg of exenatide prolonged release injection (once-weekly) vs placebo (0.9% saline)
Key inclusion and exclusion criteria	Key inclusion criteria: • Diagnosed with alcohol dependence according to the criteria of ICD-10, World Health Organization and DSM-5 (for the equivalent diagnosis of alcohol use disorder) • Age 18 - 70 years (both included) • Heavy alcohol drinking defined as having alcohol consumption over 60 g of alcohol per day (men) or 48 g of alcohol per day (women) for at least 5 days in the past 30 days prior to inclusion. Key exclusion criteria: • Severe psychiatric or somatic disease • Concomitant pharmacotherapy against alcohol dependence i.e. disulfiram, naltrexone, acamprosate and nalmefene or treatment with any of these compounds within 1 month prior to inclusion
Study type	The present study is a 26-week, double-blinded, randomized, placebo-controlled clinical trial, designed to evaluate the effects of exenatide vs. placebo in 114 patients diagnosed with alcohol dependence.
Date of first enrolment	9th of august 2017
Target sample size	114
Recruitment status	Recruiting
Primary outcome(s)	*The primary endpoint* is percent reduction in total number of heavy drinking days, defined as days with an excess intake of 60/48 grams of alcohol per day (men and women, respectively) the previous 30 days from baseline to follow-up after 26 weeks of treatment, measured by TFLB method.
Key secondary outcomes	The secondary endpoints include changes in total

Appendix A - World Health Organization Trial Registration Data Set

	alcohol consumption (g/30 days measured by TLFB), changes in number of days without alcohol consumption and PACS score, change in AUDIT score, change in DUDIT score, change in cognitive performance on the SCIP-test, change in the liver parameters gamma-glutamyltransferase (GGT), alanine aminotransferase (ALAT) and PEth. Other parameters will be mean cell volume (MCV), changes in body weight, blood pressure, pulse, overall glycaemic control parameters (HbA1c), kidney function (p-creatinine, eGFR and urine albumin/creatinine ratio) and measures of health (SF-36 and SCL-92).

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	9
	2b	All items from the World Health Organization Trial Registration Data Set	Appendix A
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	10
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	10
	5b	Name and contact information for the trial sponsor	11
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	No role
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Not relevant
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-3
	6b	Explanation for choice of comparators	2
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4-6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	4
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	4
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7-8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	4
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7-8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7-8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7-8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7-8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7-8
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-7
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	In protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	In protocol
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	In protocol
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	In protocol
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	In protocol
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	10

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	In protocol
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In protocol
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	In "Competing interests"
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	In protocol
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In protocol
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	In protocol
	31b	Authorship eligibility guidelines and any intended use of professional writers	In "Contributorship statement"
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	In protocol
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	4
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	In protocol

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.