

STUDY PROTOCOL

A randomized, double-blind, placebo-controlled, multicentre trial on the efficacy of varenicline and bupropion in combination and alone for treatment of alcohol use disorder: Protocol for the COMB study

Andrea de Bejczy^{1,2*}, Helga Lidö^{1,2}, Bo Söderpalm^{1,2}

1 Addiction Biology Unit, Psychiatry and Neurochemistry Section, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, **2** Department of Addiction and Dependency, Sahlgrenska University Hospital, Gothenburg, Sweden

* andrea.debejczy@neuro.gu.se



OPEN ACCESS

Citation: de Bejczy A, Lidö H, Söderpalm B (2024) A randomized, double-blind, placebo-controlled, multicentre trial on the efficacy of varenicline and bupropion in combination and alone for treatment of alcohol use disorder: Protocol for the COMB study. *PLoS ONE* 19(1): e0296118. <https://doi.org/10.1371/journal.pone.0296118>

Editor: James M Wright, University of British Columbia, CANADA

Received: December 22, 2022

Accepted: December 4, 2023

Published: January 11, 2024

Copyright: © 2024 de Bejczy et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: No datasets were generated or analysed during the current study. All relevant study data will be made available upon study completion and published in peer reviewed journals.

Funding: The COMB Study (COMB-B08) has been funded primarily by the Swedish Research Council (2017-00369)(BS) <https://www.vr.se/english.html>, with additional support from the Swedish Brain Foundation (F02018-0256, F02020-0220)(BS)

Abstract

Background

Alcohol Use Disorder (AUD) is a major cause of premature death, disability and suffering. Available treatments are of modest efficacy and under-prescribed so there is a pressing need for a well-tolerated and effective treatment option for AUD. Dopamine is hypothesized to be involved in the development of alcohol dependence. To challenge the low-dopamine hypothesis of addiction, this randomized, double-blind, placebo-controlled, 13-week, multi-centre clinical trial with four parallel arms is designed to evaluate the efficacy of two substances raising dopamine levels, varenicline and bupropion, alone and in combination vs. placebo on alcohol consumption in AUD. Varenicline, a partial agonist at brain nicotinic acetylcholine receptors increases dopamine release, whereas bupropion is a centrally-acting, norepinephrine-dopamine reuptake inhibitor. Varenicline is previously shown to reduce alcohol intake in individuals with AUD. We hypothesize that the effect size of a combination of two drugs affecting dopamine levels in the brain will exceed that of approved AUD therapies.

Methods

Consenting individuals with AUD will be recruited via media advertisements. Those fulfilling the eligibility criteria (N = 380) will be randomized to one of four interventions (n = 95 per arm). Treatment will comprise one week of titration (varenicline 0.5–2 mg; bupropion SR 150–300 mg) plus 12 weeks at steady state. Efficacy will be evaluated using two primary endpoints of alcohol consumption: Heavy Drinking Days and blood levels of phosphatidylethanol. Secondary objectives, exploratory and subgroup analyses will be also performed. The modified Intention-to-Treat and Per Protocol datasets will be evaluated using Analysis of Covariance. Last patient out is estimated to occur in December, 2022.

<https://www.hjarnfonden.se/om-hjarnfonden/about-hjarnfonden/>, Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (2017-0087, 2018-0079)(BS, HL, AdB) <https://www.government.se/government-agencies/swedish-alcohol-retailing-monopoly-systembolaget-aktiebolag/>, Swedish Society of Medicine (SLS-935632, SLS-786651)(HL) <https://www.sls.se/om-oss/the-swedish-society-of-medicine-in-english/>, and Söderströms-Königska Nursing Home Foundation (SLS-940846)(HL) <https://www.sls.se/vetenskap/sok-anslag/stift.-soderstrom/>. The study is also financed by grants from the Swedish state under the agreement between the Swedish Government and the county council (Region Västra Götaland), the ALF agreement (75320)(BS) <https://www.alfvastragotaland.se>, and additional support from the Department of Addiction and Dependency, Sahlgrenska University Hospital, Sweden (BS) <https://www.sahlgrenska.se/omraden/omrade-2/beroende/>. The study has received a research donation from Sobrera Pharma AB (BS) <https://sobrera.com/>. The funders had and will not have a role in the study design, data collection and analysis or decision to publish, or preparation of the manuscript. Sobrera Pharma AB have sponsored editorial support for the manuscript.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: Andrea de Bejczy and Bo Söderpalm are founders and co-owners of Sobrera Pharma AB, and Helga Lidö is co-owner of Sobrera Pharma AB. The COMB study is an academic study and all decisions about design, data collection and analysis and publications are made by the academic research group/sponsor team with PI/sponsor Bo Söderpalm. Sobrera Pharma has not produced or supplies the study drug. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

Discussion

The COMB Study aims to evaluate the efficacy of the combination of varenicline and bupropion, two drugs affecting dopamine, on alcohol consumption, and to challenge the low-dopamine hypothesis of addiction. Study Code COMB-BO8, EudraCT 2018–000048–24, Version 3.2, Lidö & deBejczy, 2020-06-16; <https://clinicaltrials.gov> identifier NCT04167306.

Introduction

Alcohol is a major contributor to the global burden of disease [1], and the average lifetime expectancy of individuals with alcohol use disorder (AUD) is estimated to be shortened by 23 years [2]. The lifetime risk of death due to alcohol-related causes (accidents and diseases) increases exponentially with alcohol consumption [3], and both abstinence and the reduction of alcohol intake can profoundly lower the risk of death in the heaviest consumers [4]. While many individuals with AUD are not motivated by the abstinence goal, they can accept the low-risk drinking level goal [5, 6]. Indeed, focus in alcohol treatment trials has shifted from abstinence as a goal to one of reducing alcohol consumption to lower risk levels [7, 8].

Currently, four medications are available for AUD: disulfiram, acamprosate, naltrexone, and nalmefene. Disulfiram is an aversion therapy with a good short-term effect albeit only in individuals who accept the abstinence goal [9]. In contrast, acamprosate, naltrexone, and nalmefene act by reducing the reinforcing effects of and/or craving for alcohol, and can be used for both abstinence and harm reduction goals [10]. Nevertheless, their effect sizes are small (Cohen's $d = 0.2$) [11–13], and all four are under-prescribed. Consequently, the treatment gap has been estimated to be over 90% [14] so new treatment options with larger effect sizes could greatly enhance treatment rates for AUD. Notably, neither disulfiram, naltrexone nor acamprosate was developed based on the neurobiology of AUD.

Alcohol interferes with the dopaminergic brain reward system. The dopamine hypothesis of drug intake and addiction focused initially on the acute rewarding, stimulative, and positive reinforcing effects of drugs of abuse, including ethanol, which appear to involve enhanced dopamine release in the nucleus accumbens (ventral striatum in man) [15, 16]. Blocking these positive reinforcing effects with pharmacological agents, therefore, was hypothesized to eliminate drug taking behaviour, which is also the assumed mechanism of action for naltrexone that at least partly prevents the dopamine releasing effect of ethanol [17]. Later, the dopamine hypothesis also included the low-dopamine state that develops upon chronic exposure to addictive drugs, and which is hypothesised to drive alcohol intake by negative reinforcement [18].

The discovery that the dopamine releasing effect of alcohol in the nucleus accumbens involves nicotinic acetylcholine receptors (nAChRs) located in the ventral tegmental area in the midbrain [19–21] triggered animal [22] and human experimental studies [23], as well as randomized controlled trials (RCTs) to investigate the use of varenicline in AUD [24, 25]. Varenicline, indicated for smoking cessation, is a selective, partial agonist of the nAChR of subtype conformation $\alpha_4\beta_2$ that elevates extracellular dopamine levels in the nucleus accumbens [26], probably by stimulating $\alpha_4\beta_2$ nAChRs located on dopaminergic cell bodies in the ventral tegmental area and/or on dopaminergic terminals in the nucleus accumbens [27]. In RCTs, varenicline has been shown to reduce alcohol intake with an effect size of approx. 0.35–0.4 (Cohen's d) [24, 25].

Although an effect size of 0.4 is larger than that for acamprosate and naltrexone, even higher effect sizes would be desirable. Treatments for comparable psychiatric public health

issues, e.g. depression and attention-deficit/hyperactivity disorder (ADHD), offer effect sizes between 0.5–0.8 [28, 29]. By combining two agents with complimentary mechanisms of action, we aim for an effect size larger than that of currently available treatments for AUD.

To complement the dopamine action of varenicline, we have chosen bupropion, an approved treatment for smoking cessation and depression. This centrally-acting norepinephrine-dopamine reuptake inhibitor elevates brain levels of norepinephrine and dopamine by blocking the action of both the norepinephrine and dopamine transporters with minimal affinity for the serotonin transporter [30]. Interestingly, in a recent preclinical study we showed that the combination of varenicline and bupropion enhanced dopamine release in rat nucleus accumbens and was associated with a synergistic, abolishing action on the alcohol deprivation effect in high alcohol-consuming rats [31], a measure considered to have a high predictive value for effects in humans [32].

Our study (COMB-BO8; NCT04167306; aka the COMB Study, full protocol can be assessed in [S5 File](#) and SPIRIT checklist in [S1 File](#)), therefore, has been designed to test the hypothesis that varenicline and bupropion, either alone or in combination, will reduce alcohol consumption in individuals with AUD. Varenicline is hypothesized to reduce alcohol consumption measured by blood phosphatidylethanol (B-PEth) levels and heavy drinking days (HDD) compared to baseline and significantly more than placebo with an expected effect size (B-PEth) of approximately 0.35 (Cohen's *d*). Combination treatment with varenicline and bupropion is hypothesized to reduce B-PEth levels and HDD compared to baseline and significantly more than placebo with an effect size (B-PEth) of 0.5–0.6 (Cohen's *d*).

In summary, the COMB study is a Phase II, randomized double-blind, placebo-controlled, 13-week, multicentre trial with four parallel groups designed to assess the efficacy and safety of varenicline and bupropion alone and in combination vs. placebo for alcohol reduction in alcohol-dependent subjects, and to challenge the low-dopamine hypothesis of alcohol addiction.

Methods

Study setting

The study will be conducted at four alcohol clinic research sites in Sweden: Gothenburg (Sponsor and Coordinating Centre), Stockholm, Linköping, and Region Skåne. The study sites and principal investigators are listed in [S1 Table](#). A central laboratory appointed by the Coordinating Centre will analyse B-PEth (both a primary outcome and an eligibility measure) from all study sites. All safety parameters and secondary outcome measures (described in “Measures”) will be analysed at each site according to site-specific practice.

The study period comprises a total of nine visits to a research site; one screening visit to assess eligibility followed, within 10 days, by randomization, 1 week of titration; and, bi-weekly visits until the end of treatment by Visit 9. Subjects will be followed up for safety on days 7 and 30 by phone call. Treatment duration comprises 1 week of titration plus 12 weeks of steady-state treatment.

Screening and eligibility criteria

Inclusion criteria. Subjects aged 25–70 years at screening and diagnosed with moderate and severe AUD according to DSM-V (meeting ≥ 4 out of 11 criteria) must provide signed informed consent and have a blood alcohol level $< 0.1\%$ (0.1 g/L) at signing. Subjects must also have B-PEth levels of ≥ 0.5 $\mu\text{mol/L}$ at screening (Visit 1) and continuous, high alcohol consumption over the last 3 months prior to screening (defined by ≥ 2 HDD per week during a typical week). They should also be available for contact by phone, and be able to read and write in Swedish.

Exclusion criteria. Subjects with total abstinence between the screening and randomization visits will be excluded, as will those who received alcohol withdrawal treatment within 30 days of study initiation or any pharmacological (including, but not exclusive to, varenicline, bupropion, disulfiram, acamprosate, naltrexone, nalmefene, baclofen, topiramate, ondansetron, mirtazapine, methylphenidate, dexamphetamine, atomoxetine, pregabalin, buprenorphine, and methadone) or non-pharmacological treatment affecting alcohol consumption within 3 months of study initiation and during the study period that could affect alcohol consumption. Subjects using antidepressants, opioid analgesics, benzodiazepines, zopiclone, zolpidem, hydroxyzine, alimemazine, propiomazine or other sedatives continuously, but not sporadically, will be excluded, as well as those using any concurrent medication that may affect the results of the trial or compromise the safety of the subjects (the SmPCs for any concurrent medication will be checked for possible interactions with study drugs).

Subjects with laboratory hepatic values > 3 times the upper limit of the normal range, creatinine clearance < 30 ml/min or other clinically significant abnormalities in the screening clinical laboratory values; and/or, blood pressure $\geq 180/110$ at screening will be excluded, as will any pregnant or breast-feeding subject or premenopausal female not using the allowed contraceptive methods (oral contraceptive, copper/hormonal intrauterine contraceptive device or subcutaneous implants).

Subjects with diabetes mellitus types 1 or 2 who require insulin treatment; any current psychiatric or somatic disorder or condition that may affect assessments or compromise their safety; Adult ADHD Self-Report Scale (ASRS; v. 1.1) Part A score ≥ 4 in the marked cut-off section; Montgomery Åsberg Depression Rating Scale (MADRS) score ≥ 20 ; current, non-mild depression; suicidality; current illicit drug use based on urine-toxicity testing and a Drug Use Disorder Identification Test (DUDIT); a history of delirium tremens or abstinence-induced seizures within 5 years of study initiation; non-alcohol-induced epilepsy/seizures (lifetime); severe sleep disturbances; a need for alcohol detoxification; living conditions not appropriate to fulfil study requirements; herbal drugs/tea and supplementations use that might affect outcome(s) or safety; previous randomization in this trial or participation in another trial within 3 months of enrolment into this trial; and, additional factors that render the subject unable to complete the study (as judged by the investigator) will be excluded.

No concomitant care for AUD will be allowed, although somatic care will be allowed if this includes no prohibited medications. Non-eligible subjects will be referred to an appropriate alcohol clinic/healthcare center or, if required due to a medical condition other than alcohol dependence or an alcohol-related condition, may be referred to an appropriate clinic.

Interventions, dosages, and administration

Varenicline tartrate (Pfizer Innovations, USA) will be used to manufacture varenicline, the investigational medicinal product (IMP 1). The dose of varenicline will be escalated from 0.5 to 2 mg daily during the first week as follows: 0.5 mg varenicline (or corresponding placebo capsules) once daily on days 1–3 and 0.5 mg varenicline (or corresponding placebo capsules) twice daily on days 4–7, after which 1 mg varenicline (or corresponding placebo capsules) will be administered twice daily (1 mg x 2 p.o. daily) on days 8–91 of treatment (visits 3–9). No dose adjustment of varenicline will be allowed.

Bupropion Sandoz SR (Slow Release formulation; Sandoz AS, Switzerland) will be used to manufacture bupropion (IMP 2). The dose of bupropion will be escalated from 150 to 300 mg daily during the first week as follows: bupropion SR 150 mg or corresponding placebo capsules once daily on days 1–7, after which 150 mg bupropion SR or corresponding placebo capsules

will be administered twice daily (150 mg x 2 p.o. daily) on days 8–91 of treatment (visits 3–9). No dose adjustment of bupropion will be allowed.

Apoteket Produktion & Laboratorier (APL) will manufacture the IMPs using a procedure to encapsulate tablets, and package and label them according to Good Manufacturing Practice (GMP). The IMPs will be packaged in unit-dose blister packs and distributed according to GMP. The formulation of the IMPs and placebo will be indistinguishable as regards appearance, shape, smell, and taste.

Subjects will receive varenicline or bupropion SR on seven occasions (visits 2, 3, 4, 5, 6, 7 and 8). Treatment will start the day after randomization and after the first week of titration, varenicline and bupropion SR will be distributed to subjects every second week.

As reported previously [24, 25], 12 weeks of steady-state treatment duration are estimated to be sufficient to detect tentative efficacy effects (measured by B-PEth and HDD) whilst concurrently manageable for study participants, thereby also optimizing study compliance.

Adherence strategies and procedures for monitoring adherence to protocol include the return of IMP and blister packs by subjects, pill count, and IMP plasma concentration analysis at visits 4 and 6.

Study discontinuation

Trial participation is voluntary and subjects can choose to discontinue at any point. The exclusion criteria apply throughout the entire study period and subjects may be withdrawn from the study at the discretion of the investigator if judged non-compliant with study procedures or for safety concerns. The treatment code will not be broken except in medical emergencies when appropriate management of the subject will necessitate knowledge of the treatment randomization.

Should any adverse event (AE) that may affect the benefit-risk balance be reported, subjects may be unblinded to enable a decision to abort the trial. Subjects will be excluded from the analysis set if an AE that is considered to affect the assessments and/or the result of the trial is reported whether or not subject safety is not compromised. Serious adverse events (SAEs) will be followed up.

At pre-term termination, subjects will undergo an end-of-trial assessment.

Measures

Primary objectives and outcomes. The primary objective will be to evaluate the effects of varenicline and bupropion alone and in combination in reducing alcohol consumption in subjects with AUD using two primary efficacy endpoints: (i) alcohol consumption as measured by blood levels ($\mu\text{mol/L}$) of B-PEth, and (ii) subjective alcohol consumption as measured by HDD using the timeline follow-back (TLFB) procedure and defined as ≥ 5 units for men and ≥ 4 units for women with 1 unit defined as 14 grams of pure alcohol.

Subjective alcohol consumption measured by HDD is a FDA-approved, clinical trial outcome variable for alcohol consumption, whilst the objective alcohol biomarker B-PEth that occurs in red blood cells in humans in the presence of alcohol [33] is a validated biomarker for alcohol consumption. The sensitivity and specificity of B-PEth are 94.5% and 100%, respectively [34], both of which are superior to carbohydrate deficient transferrin (CDT), an indirect alcohol marker for moderate to heavy drinking for approx. 2–4 weeks [35], and gamma glutamyltransferase (GGT), an indirect alcohol marker for moderate to heavy drinking for approx. 4 weeks [24, 35]. The half-life of B-PEth is approx. 1 week, and the detection window is days to several weeks [36–38].

Blood levels of B-PEth will be calculated as the mean value over the 8-week, steady-state, active treatment period (Visit 4 to Visit 8) vs. baseline. Data will be analysed as the within patient difference. Visits 9 and 3 will be omitted in the analysis period due to the tendency of drop-out and non-compliance at the end of a study period (Visit 9) and due to titration of IMP and the optimal timeframe of B-PEth analysis (Visit 3). Additionally, the B-PEth vs. baseline levels analysis will be performed at all visits.

The HDD number by 14 days is defined as a mean over the 8-week, steady-state, active treatment period (Visit 4 to Visit 8), in 14-day period measures vs. baseline. The baseline mean of HDD by 14 days will be obtained at the screening visit, where the number of HDD over the last 30 days will be recorded. Additionally, the HDD analysis vs. baseline levels will be performed at all visits.

Secondary objectives and outcomes. The following secondary objectives will be evaluated: (i) the effects of varenicline and bupropion alone and in combination in reducing alcohol consumption in subjects with AUD using the indirect alcohol markers CDT and GGT, self-reported alcohol consumption using TLFB, the mean grams of alcohol per day, and, the number of drinking days, drinks per drinking days and abstaining days; (ii) the Total score of Alcohol Use Identification Test (AUDIT); (iii) alcohol craving measured by a Visual Analogue Scale (VAS); (iv) nicotine use as measured by the nicotine saliva marker cotinine; (v) the Temporal Experience of Pleasure Scale (TEPS); (vi) the Continuous Performance Test + Activity test (CPTA); (vii) and, the relationships between the aforementioned outcome measures and plasma drug concentrations of bupropion and varenicline.

Additional analyses. Efficacy will be evaluated in relation to the plasma drug concentrations of varenicline and bupropion, and for compliance. Subgroup analyses will be performed on the World Health Organization consumption level categories (defined in “Subgroup analyses”), the severity of AUD, and family history of alcohol problems. Data will also be analysed to identify characteristics for responders and to evaluate placebo effects in early responders. Additionally, dopamine-related variables and genetic factors will be analysed.

Study assessments. The following parameters will be measured/determined: breath alcohol concentration (BrAc) to ensure sobriety during both informed consent and cognitive testing (CPTA); demographics (gender, date of birth, civil status, number of children, and education level); alcohol-specific factors (answers to questions specifically related to quantity, frequency, family history of alcohol dependence, age of alcohol debut); nicotine-specific factors (answers to questions specifically related to quantity, frequency, age of debut); AUD diagnosis meeting ≥ 4 criteria according to DSM-V by structured interview; medical history (i.e., previous psychiatric and somatic disorders); routine physical examination, including heart and lung auscultation and palpation of abdomen; and, vital signs (blood pressure [systolic and diastolic] and heart rate after 10 minutes of rest, weight, and height). The study assessments and timelines are shown in Fig 1, and the scales and questionnaires are summarised in Fig 2.

Safety endpoints. The following parameters will be monitored as safety endpoints: levels of aspartate aminotransferase, alanine aminotransferase, prothrombin complex, creatinine glucose, Na^+/K^+ , haemoglobin, the leukocyte plasma count, the thrombocyte particle concentration, high-sensitive C-reactive protein, human chorionic gonadotropin (for fertile women), urine toxicology, and the MADRS and DUDIT self-reporting scales for depression and drug abuse, respectively, according to the schedule shown in Fig 1.

Study and participant timelines

The timeline for the COMB Study is depicted in Fig 3. All study visits are summarized Fig 1. At all visits and follow ups, the study outcome measures HDD (according to TLFB) will be

Hb		√								√		
LPC		√								√		
TPC		√								√		
hsCRP (also outcome measure)		√								√		
U-hCG		√	√							√		
U-tox		√	√							√		
MADRS		√			√					√		
Timepoint	Ph Scr	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	FU +7	FU +30
INTERVENTION – STUDY MEDICATION												
Treatment duration			√	√	√	√	√	√	√	√		
Study medication distribution			√	√	√	√	√	√	√			
Drug accountability by pill count				√	√	√	√	√	√	√		
Drug plasma concentration bupropion					√		√					
Drug plasma concentration varenicline					√		√					

Fig 1. SPIRIT schedule of enrolment and summary of study assessments in the COMB study. AE = adverse event; ALT = alanine aminotransferase; ASRS = adult ADHD self-report scale; AST = aspartate aminotransferase; AUD = alcohol use disorder; AUDIT = alcohol use disorder identification test; B-PEth = blood phosphatidylethanol; CDT = carbohydrate deficient transferrin; CPTA = continuous performance test + activity; DUDIT = drug use disorder identification test; FU = follow up; GGT = gamma-glutamyltransferase; Hb = haemoglobin; hsCRP = high sensitive C-reactive protein; LPC = leukocyte plasma count; MADRS = Montgomery Åsberg depression rating scale; Na⁺/K⁺ = sodium/potassium; PC = prothrombin complex; Ph = phone; QF = quantity frequency; SAE = serious adverse event; Scr = screening; TEPS = temporal experience of pleasure scale; TLFB = timeline follow-back; TPC = trombocyte particle concentration; U-hCG = urine human chorionic gonadotropin; U-tox = urine toxicology; VAS = visual analogue scale. *If judged as necessary by medical physician or study participant.

<https://doi.org/10.1371/journal.pone.0296118.g001>

collected; all AEs will be documented and evaluated as regards any potential associations with the IMPs/study procedure and level of seriousness (potential SAEs and suspected unexpected serious adverse reactions will be evaluated and treated according to standard of care at the investigating centre); and, concomitant medications will be documented and any potential impact on safety and study results evaluated. At all visits, blood for B-PEth analysis will be collected and vital signs will be defined.

Scale	Measurement	Description	References
Adult ADHD Self-Report Scale (ASRS)	Eligibility	A self-report instrument symptom checklist for ADHD screening	[39]
Alcohol Use Disorders Identification Test (AUDIT)	Outcome	A 10-item self-report questionnaire for alcohol-problem screening	[40] [41]
Drug Use Disorder Identification Test (DUDIT)	Eligibility and Safety	An 11-item self-report questionnaire to evaluate current drug use	[42]
Montgomery-Asberg Depression Scale (MADRS)	Eligibility and Safety	A 10-item self-rating scale for depressive symptoms	[43]
Temporal Experience of Pleasure Scale (TEPS)	Outcome	A 17-item scale with anticipatory and consummatory components of the experience of pleasure	[44]
Timeline Follow-back (TLFB)	Outcome	A retrospective method for assessing self-reported alcohol consumption.	[45]

Fig 2. Scales and questionnaires used in the COMB study.

<https://doi.org/10.1371/journal.pone.0296118.g002>

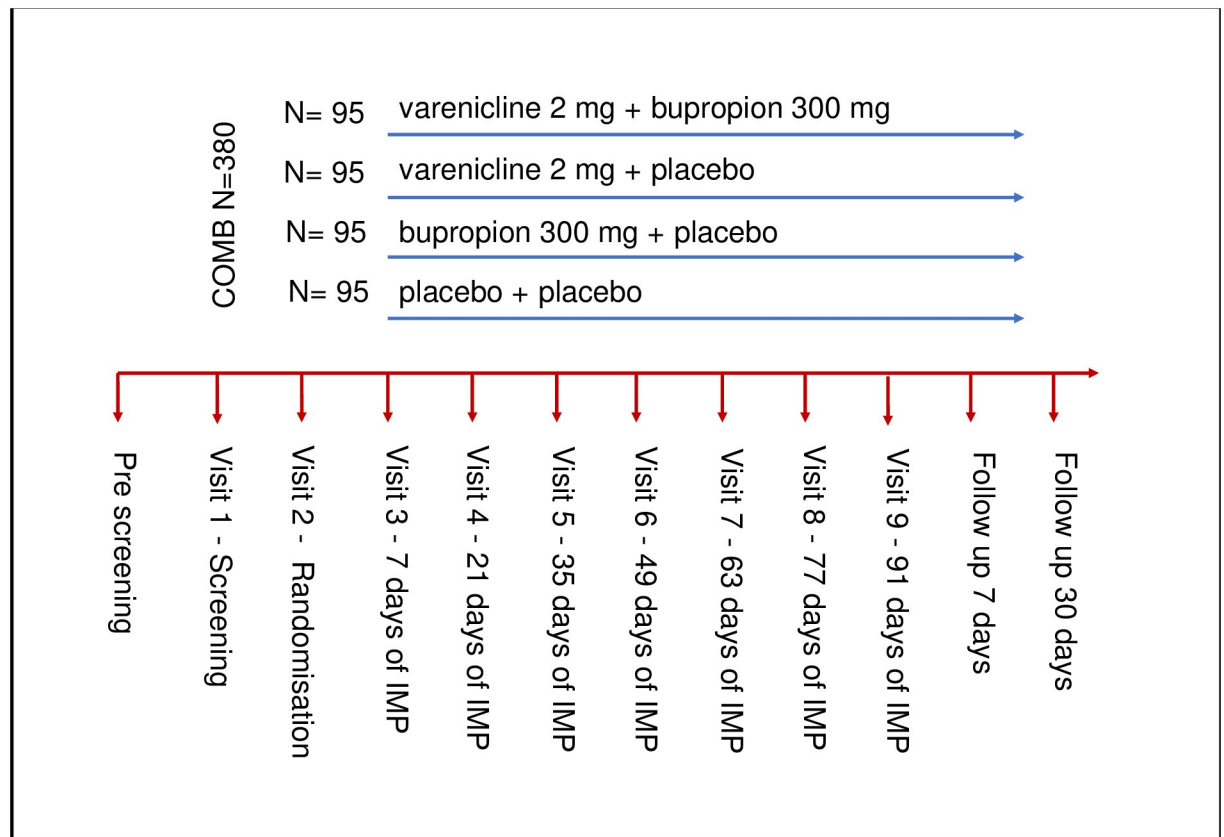


Fig 3. Treatment arms and schematic time line for study visits.

<https://doi.org/10.1371/journal.pone.0296118.g003>

Potential participants who respond to media advertisements will be identified via pre-screening by phone. At Visit 1 (screening/baseline visit, acceptable range $< +14$ days from pre-screening), subjects' breath alcohol levels will be evaluated, after which subjects must provide a signed, written Informed Consent Form before eligibility criteria will be evaluated; and, the study outcome variables (except for B-Peth) and safety parameters will also be evaluated. At Visit 2 (randomization visit, accepted range $< +10$ days from screening visit), eligibility criteria will be evaluated and subjects will receive a patient emergency card. Visits 3, 5 and 7 comprise the core battery visits (accepted range ± 3 days from scheduled visit date). At Visit 4 (core battery, IMP concentration and safety visit, accepted range ± 3 days from scheduled visit date), all safety parameters will be evaluated and blood samples taken to determine the IMP concentrations. At Visit 6 (core battery and IMP concentration, accepted range ± 3 days from scheduled visit date), blood samples will be taken to determine IMP concentrations. At Visit 8 (end of analysis visit, accepted range ± 3 days from scheduled visit date), study outcome variables and safety parameters will be evaluated. At Visit 9 (end of treatment/medical visit, accepted range ± 3 days from scheduled visit date), subjects will undergo a medical examination by the physician. Subjects will be followed up by phone 7- and 30-days post end of treatment.

The COMB Study comprises a double-blind phase with nine visits in total over 13 weeks (91 days). Days 0–7 indicate the titration of dosing.

Sample size and recruitment

A total of 380 eligible subjects (95 subjects per treatment arm) will be recruited via adverts in newspapers, television, and social media for randomization into the study. To ensure adequate recruitment and compliance to study protocol, two advertising periods per year will be performed. Potential subjects will be evaluated in batches to maximize enrolment. Several different media will be used to maximize the reach of potential subjects of different genders and ages.

The power analysis for total N is based on two independent primary hypotheses: (i) B-PEth differs significantly between varenicline and bupropion alone and in combination vs. placebo, and (ii) the proportion of HDD differs significantly between varenicline and bupropion alone and in combination vs. placebo. With $\alpha = 0.025$, $1 - \beta = 0.80$ and considering the outcome with the highest predicted sample size, total N has been estimated to be 360. The drop-out rate is estimated to be approx. 20% [24] between screening and randomization, and approx. 6.5% for randomization of subjects included in the modified ITT population. Thus, a sample size of approximately 380 subjects will be randomized.

Randomization, allocation and blinding

Apotekets Produktion och Laboratorium, the appointed manufacturer of the IMPs, will package and label varenicline and placebo, and bupropion and placebo as two separate IMP kits. The manufacturer will use a blinding procedure (based on the internal standard operating procedure of the manufacturer), where the respective treatment kits for IMP 1 and IMP 2 will have been given a unique, sequentially numbered randomization number, generated randomly. Randomization will be performed according to block randomization (block-size 8). Each IMP will be allocated blinded to study sites. Subjects who meet the eligibility criteria will be randomly assigned to one of four treatment groups by the site study investigator in a 1:1:1:1 ratio to one of the following treatments: Varenicline + Placebo; Bupropion + Placebo; Varenicline + Bupropion; or Placebo + Placebo (Fig 3). Treatment will be blinded to subjects, investigators, and study personnel. Data will be analysed blinded by a statistical data analyst before the code is broken.

Opaque, sealed emergency envelopes containing information about each subject's treatment will be kept at the Coordinating Centre.

Data collection

All scales and questionnaires that will be used in the COMB Study to evaluate the eligibility and outcome measures (Fig 2) are validated and have high reliability [39–46]. Study personnel collecting trial data will be proficient in all study assessments and procedures. To enhance subject retention, all site visits will be planned at the randomisation visit and subjects will be contacted by phone at any deviation from the visit plan. All study personnel will be proficient in collecting and handling samples for blood, urine, and saliva testing. The laboratory tests are documented in “Measures” and Fig 1. Subjects who discontinue the study will be followed up by phone after 7 days and 30 days, according to protocol. Data collection forms will be written in Swedish (available upon request).

Data management

All study-specific data will be recorded coded in the electronic Case Report File (eCRF), available only to delegated study personnel educated in data entry, password protected, and stored in secure servers. Data entry will be performed at each site in association with data collection.

Data quality will be ensured by built in range and data validation checks. Data are audit trailed and monitored and signed by the investigator at each site.

Data management will be performed according to the Data Management Plan ([S2 File](#)). Sanity checks will be run on all included subjects, approximately once per month. A Data Manager will be responsible for database lock that will be performed after clean file has been declared and the Principal Investigator has downloaded an independent copy of the eCRF data for archiving.

Statistical analysis

The mean values of each variable (primary and secondary) for the different treatment groups will be compared hierarchically using Analysis of Covariance (ANCOVA). Testing between treatments is performed hierarchically so no adjustment for multiple testing will be performed. Sensitivity to missing data will be evaluated by imputation methods, and robustness of results will be evaluated by sensitivity analyses as per the Statistical Analysis Plan (SAP; [S3 File](#)).

Exploratory analyses. Exploratory analyses will be performed on the impact of demographic data on the aforementioned primary and secondary endpoints; the relationship between the primary and secondary outcome measures; and, the effects of the primary and secondary outcome measures in relation to blood drug concentrations of bupropion and varenicline. Different models and *ad hoc* analyses will be performed, as specified in the SAP.

Subgroup analyses. Subgroup analyses will be performed on: gender; heredity (defined by family history positive); alcohol consumption levels at screening (defined by the World Health Organization consumption level categories: 0–40 g/day, 40–60 g/day, 60–100 g/day and > 100 g/day); DSM diagnostic criteria for severe AUD at screening (defined by ≥ 6 criteria); early debut of alcohol consumption (age ≤ 12 years) as a proxy for early-onset AUD; responders/non-responders (defined by either a downward shift of two WHO consumption level categories measured by total alcohol consumption vs. baseline or a $\geq 50\%$ reduction in B-PEth levels vs. baseline); early responders (defined by either a $\geq 50\%$ reduction in B-PEth levels or a $\geq 50\%$ reduction in HDD or a $\geq 50\%$ reduction in total alcohol consumption between Visit 1 [screening] and Visit 2 [randomization/start IMP]). These analyses will be performed as per the SAP using different models and *ad hoc* analyses and divided by subgroups as defined.

Analysis sets. The modified ITT analysis set will include all study subjects who complete both the screening and randomization visits and report having taken at least one dose of the randomized study drugs, and they will be analysed according to their randomized study drug.

The Per Protocol analysis set will constitute all subjects from the ITT population considered to be “completers”. A completer is defined as a subject who has taken the IMP at least 80% of the planned number of days of the active treatment period and provided successful outcome measures of B-PEth and/or TLFB at screening and visits 2, 4, 6 and 8.

The safety analysis set will include all subjects who report having taken at least one dose of randomized study drug and for whom any post-dose data is available until Visit 8 (safety visit). Subjects will be analysed according to the actual treatment.

Monitoring

Data monitoring and auditing. The study will be monitored and independently audited according to the monitoring plan ([S4 File](#)) by a monitor independent of the Sponsor and with no competing interests. All monitoring will be documented and trailed in the eCRF and the monitor will report to the Sponsor. An independent audit will be performed by CGP Consult. No interim analyses will be performed.

Harms. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All AEs that are observed by study personnel or reported by the subject during the trial will be recorded with the following information: description, date of onset and end date, severity, assessment of relatedness to trial medication and action taken, and whether or not it is attributable to trial medication. Adverse events will be assessed and authorized by a responsible medical physician. If considered to related to the trial medication, the AEs will be followed either until resolution or the event is considered to be stable. All SAEs will be reported on the SAE Reporting Form to the Sponsor or delegate within stipulated time frame. All Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported by the Sponsor to the relevant Competent Authority, the Ethics Committee, and all participating investigators, and reported via the Swedish Medical Products Agency to the Eudra Vigilance system within stipulated time frame.

Ethics and dissemination

Research ethics approval, protocol amendments, and consent. The study will be performed in accordance with the study protocol, the WMA Declaration of Helsinki (October, 2013), GCP principles [i.e., ICH-GCP E6(R2)], all applicable regulatory requirements (i.e., “Läkemedelsverkets föreskrifter”, LVFS 2011:9), and the EU General Data Protection Regulation (GDPR) 2016/679 privacy legislation. Ethical approval has been obtained from the Swedish Ethical Review Authority (EPM; D.nr. 431–18, 2018-06-18, amendment 2019-07-05/2019-11-26 D.nr. 2019–03559, amendment 2020-10-21 D.nr. 202004924, amendment 2022-01-14 D.nr 2021-06785-02). Approval has been obtained from the Swedish Medical Product Agency (MPA; EudraCT 2018–000048–24, amendment 2019-07-31 D.nr.5.1-2019-50751, amendment 2022-01-31 D.nr. 5.1-2021-101134). All subjects must provide written informed consent, obtained by delegated medical physician, to enter the study and for collection of all data relevant to the study, as per protocol. The full protocol and SAP, including any amendments, can be accessed in [S3](#) and [S5](#) Files, respectively.

Confidentiality. Participation in the COMB study will be recorded in a medical chart according to the Swedish Patient Data Act (2008:355) and informed consent will comply with relevant data protection, i.e., the EU General Data Protection Regulation (GDPR) 2016/679 privacy legislation. Study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. The identities of the subjects will not be disclosed in any stored data.

Ancillary and post-trial care. Trial participants will be insured by the Swedish Patient Injury Act and the Swedish Pharmaceutical Insurance (Läkemedelsförsäkringen, see <http://lff.se/>).

Dissemination policy. Last patient out is estimated to occur in December, 2022 and study results will be submitted for publication in a scientific, peer-reviewed journal during 2023/2024. All authors will fulfil the ICMJE guidelines for authorship, and will also agree to be accountable for all aspects of the work.

Discussion

In this study we have chosen varenicline and bupropion, two drugs with different but complementary mechanisms of action to enhance dopamine levels in the brain, to challenge the low-dopamine hypothesis of alcohol addiction. By combining drugs with dopamine action, we seek to exceed the effect size of currently available treatments for AUD.

In AUD, human studies indicate that both pre- and post-synaptic aspects of basal dopamine neurotransmission are reduced [47, 48]. The resultant compromised dopamine system [49] may be genetically determined and/or develop as an adaptation to chronic alcohol use [50].

Furthermore, reduced dopamine neurotransmission has been associated with increased alcohol intake, as well as increased craving and alcohol-cue reactivity, both in animal [51, 52] and human [53] studies. The low-dopamine state is hypothesised to drive alcohol intake by negative reinforcement [18].

Trials that investigate the beneficial effects of enhancing dopamine neurotransmission are sparse, and studies that apply agents to increase endogenous dopamine levels are essentially lacking, except for those using varenicline. Moreover, the exact mechanism(s) underlying the observed effects of varenicline on AUD in RCTs [24, 25] have not been established. A reasonable hypothesis, however, is that they derive from its dopamine-elevating effect, and that the reported studies represent proof-of-concept that elevating central dopamine levels reduces alcohol intake.

We assume that further dopamine elevation is likely to enhance the effect and, indeed, recent evidence from animal studies that co-administration of varenicline and the dopamine-noradrenaline reuptake inhibitor bupropion enhances both the dopamine output in the nucleus accumbens and the reduction of the alcohol deprivation effect produced by varenicline supports this assumption [31].

The mechanisms by which varenicline and bupropion produce their limited dopamine elevations cannot be overridden by adding more drug of the respective drug, which limit the risk of abuse. Both drugs, however, are well tolerated and safe across various populations, and have been recommended in patients with psychiatric or addictive comorbidity [54].

In conclusion, the present study aims to challenge the low-dopamine hypothesis of AUD by pharmacologically mitigating the low-dopamine state that is thought to drive alcohol intake, whilst simultaneously evaluating the efficacy of the combination of varenicline and bupropion, which have complimentary mechanisms of action on the brain dopamine system, on alcohol consumption in AUD.

Strengths

The COMB study is scientifically sound as regards power and design with biweekly data collection and an objective alcohol consumption blood marker (B-PEth) as one of the primary outcome measure. Moreover, B-PEth as an inclusion criterion will also ensure a high level of recent alcohol consumption. Additional design strengths include a sufficient time window between pre-screening, screening, and randomisation as this will reduce recruitment procedure discrepancies between sites, and recruiting potential trial participants in batches via advertisements to maintain the recruitment timeline. Since no psychotherapy will be administered, this will also strengthen the focus on the efficacy of the IMP under investigation.

Importantly, this study protocol will enable us to comprehensively evaluate the efficacy of varenicline and bupropion, either alone or in combination, in the AUD population. Alcohol is a major cause of premature death and disability and, with few available treatments that are of moderate efficacy, the need for new treatment options is pressing. Expanding our understanding of the mechanisms underlying alcohol dependence is also vital so in addition to the efficacy outcome, the secondary and explorative study outcomes will also shed light on the dopamine deficiency theory, enabling us to interrogate the dopamine hypothesis associated with addictive diseases. Notably, this study will be the first to use B-PEth for alcohol intake as a primary outcome variable in order to increase the chance of detecting treatment effects that may drive future study guidelines in AUD.

Limitations

The COMB Study has been conducted during the Covid-19 Pandemic (2020–21). Although local restrictions associated with the pandemic have influenced study procedures in some

instances, our overall assessment is that the COMB Study has been performed according to protocol.

In July 2021, Pfizer globally withdrew varenicline (Champix[®], Chantix[®]) from the market due to levels of N-nitroso-varenicline reported as being above acceptable limits. Since the IMP 1 for the COMB Study has been produced in batches due to sustainability factors, the final batch of IMP 1 produced in November 2021 contained the varenicline equivalent, Apo-Varenicline (Apotex[®]), with no reports to date of nitrosamine levels that would be expected to affect safety or study results. The last batch of IMP has also been subject to a limited shelf life due to distribution issues with varenicline, although this has had no negative impact on the study.

In summary, the COMB Study aims to be of value to the international AUD community not only by testing/validating a new pharmacological treatment strategy for AUD for which there is a high clinical need, but also in challenging the long-standing, low-dopamine hypothesis of addiction, which may lead to novel AUD treatment strategies.

Supporting information

S1 File. SPIRIT checklist for the COMB study.

(PDF)

S2 File. Data management plan for the COMB study.

(PDF)

S3 File. Statistical analysis plan for the COMB study.

(PDF)

S4 File. Monitoring plan for the COMB study.

(PDF)

S5 File. Protocol for the COMB study.

(PDF)

S1 Table. Sites participating in the COMB study.

(PDF)

Acknowledgments

We acknowledge the help of all site personnel, including investigators Prof. Johan Franck, MD, PhD (Stockholm Centre for Dependency Disorders, SLSO), Prof. Anders Håkansson, MD, PhD (Psychiatry Malmö) and Prof. Markus Heilig, MD, PhD (Linköping University Hospital), with special thanks to Barbro Askerup and Cecilia Nilsson-Wallmark for study coordination. We also acknowledge the help of Statistikkonsulterna Jostat and MR Sample AB for statistic support; MediCase AB for data management/eCRF support; Opatas AB for CPTA support; Christina Harthon and Gothia Forum for monitoring support; Hellgren GCP Consulting for regulatory support and study auditing; Lisa Walther at Lund University for B-PETH expertise; and, the Department of Addiction Sahlgrenska University Hospital and the Institute of Neuroscience and Physiology, Section of Psychiatry and Neurochemistry Sahlgrenska Academy, Gothenburg University. We acknowledge Dr Grażyna Söderbom at Klipspringer AB for editorial support. Our sincere thanks go out to all participants who enrolled in the study for their time, commitment, and effort.

Author Contributions

Conceptualization: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Data curation: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Formal analysis: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Funding acquisition: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Investigation: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Methodology: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Project administration: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Resources: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Software: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Supervision: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Validation: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Visualization: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Writing – original draft: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

Writing – review & editing: Andrea de Bejczy, Helga Lidö, Bo Söderpalm.

References

1. WHO. WHO. Global status report on alcohol and health. 2018. <https://www.who.int/publications/i/item/9789241565639>
2. Lesch OM, Lesch E, Dietzel M, Mader R, Musalek M, Walter H, et al. [Chronic alcoholism—alcohol sequelae—causes of death]. *Wien Med Wochenschr.* 1986; 136(19–20): 505–515.
3. Rehm J. The risks associated with alcohol use and alcoholism. *Alcohol Res Health.* 2011; 34(2): 135–143. <https://doi.org/10.1111/j.1360-0443.2010.03167.x> PMID: 22330211
4. Rehm J, Roerecke M. Reduction of drinking in problem drinkers and all-cause mortality. *Alcohol Alcohol.* 2013; 48(4): 509–513. <https://doi.org/10.1093/alcalc/agt021> PMID: 23531718
5. Hodgins DC, Leigh G, Milne R, Gerrish R. Drinking goal selection in behavioral self-management treatment of chronic alcoholics. *Addict Behav.* 1997; 22(2): 247–255. [https://doi.org/10.1016/s0306-4603\(96\)00013-5](https://doi.org/10.1016/s0306-4603(96)00013-5) PMID: 9113218
6. Heather N, Adamson SJ, Raistrick D, Slegg GP, Team UR. Initial preference for drinking goal in the treatment of alcohol problems: I. Baseline differences between abstinence and non-abstinence groups. *Alcohol Alcohol.* 2010; 45(2): 128–135. <https://doi.org/10.1093/alcalc/agn096> PMID: 20130149
7. Nutt DJ, Rehm J. Doing it by numbers: a simple approach to reducing the harms of alcohol. *J Psychopharmacol.* 2014; 28(1): 3–7. <https://doi.org/10.1177/0269881113512038> PMID: 24399337
8. Mann K, Bladstrom A, Torup L, Gual A, van den Brink W. Extending the treatment options in alcohol dependence: a randomized controlled study of as-needed nalmefene. *Biol Psychiatry.* 2013; 73(8): 706–713. <https://doi.org/10.1016/j.biopsych.2012.10.020> PMID: 23237314
9. Wright C, Moore RD. Disulfiram treatment of alcoholism. *Am J Med.* 1990; 88(6): 647–655. [https://doi.org/10.1016/0002-9343\(90\)90534-k](https://doi.org/10.1016/0002-9343(90)90534-k) PMID: 2189310
10. Socialstyrelsen. Kostnader för alkohol och narkotika, beräkningar av samhällets direkta kostnader. 2010. <http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/17961/2010-3-15.pdf>.
11. Rosner S, Hackl-Herrwerth A, Leucht S, Vecchi S, Srisurapanont M, Soyka M. Opioid antagonists for alcohol dependence. *Cochrane Database Syst Rev.* 2010;(12): CD001867. <https://doi.org/10.1002/14651858.CD001867.pub3> PMID: 21154349
12. Soyka M, Rosner S. Emerging drugs to treat alcoholism. *Expert Opin Emerg Drugs.* 2010; 15(4): 695–711. <https://doi.org/10.1517/14728214.2010.500811> PMID: 20560783
13. Jonas DE, Amick HR, Feltner C, Bobashev G, Thomas K, Wines R, et al. Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis. *JAMA.* 2014; 311(18): 1889–1900. <https://doi.org/10.1001/jama.2014.3628> PMID: 24825644

14. Kohn R, Saxena S, Levav I, Saraceno B. The treatment gap in mental health care. *Bull World Health Organ.* 2004; 82(11): 858–866. PMID: [15640922](https://pubmed.ncbi.nlm.nih.gov/15640922/)
15. Engel J, Carlsson A. Catecholamines and behavior. *Curr Dev Psychopharmacol.* 1977; 4: 1–32. PMID: [23246](https://pubmed.ncbi.nlm.nih.gov/23246/)
16. Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev.* 1987; 94(4): 469–492. PMID: [3317472](https://pubmed.ncbi.nlm.nih.gov/3317472/)
17. Valenta JP, Job MO, Mangieri RA, Schier CJ, Howard EC, Gonzales RA. μ -opioid receptors in the stimulation of mesolimbic dopamine activity by ethanol and morphine in Long-Evans rats: a delayed effect of ethanol. *Psychopharmacology (Berl).* 2013; 228(3): 389–400.
18. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology.* 2001; 24(2): 97–129. [https://doi.org/10.1016/S0893-133X\(00\)00195-0](https://doi.org/10.1016/S0893-133X(00)00195-0) PMID: [11120394](https://pubmed.ncbi.nlm.nih.gov/11120394/)
19. Soderpalm B, Lof E, Ericson M. Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. *Pharmacopsychiatry.* 2009; 42 Suppl 1: S87–94. <https://doi.org/10.1055/s-0029-1220690> PMID: [19434560](https://pubmed.ncbi.nlm.nih.gov/19434560/)
20. Soderpalm B, Ericson M. Neurocircuitry involved in the development of alcohol addiction: the dopamine system and its access points. *Curr Top Behav Neurosci.* 2013; 13: 127–161. https://doi.org/10.1007/7854_2011_170 PMID: [22094880](https://pubmed.ncbi.nlm.nih.gov/22094880/)
21. Soderpalm B, Lido HH, Ericson M. The glycine receptor—a functionally important primary brain target of ethanol. *Alcohol Clin Exp Res.* 2017; 41(11): 1816–1830. <https://doi.org/10.1111/acer.13483> PMID: [28833225](https://pubmed.ncbi.nlm.nih.gov/28833225/)
22. Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci U S A.* 2007; 104(30): 12518–12523. <https://doi.org/10.1073/pnas.0705368104> PMID: [17626178](https://pubmed.ncbi.nlm.nih.gov/17626178/)
23. McKee SA, Harrison EL, O'Malley SS, Krishnan-Sarin S, Shi J, Tetrault JM, et al. Varenicline reduces alcohol self-administration in heavy-drinking smokers. *Biol Psychiatry.* 2009; 66(2): 185–190. <https://doi.org/10.1016/j.biopsych.2009.01.029> PMID: [19249750](https://pubmed.ncbi.nlm.nih.gov/19249750/)
24. de Bejczy A, Lof E, Walther L, Guterstam J, Hammarberg A, Asanovska G, et al. Varenicline for treatment of alcohol dependence: a randomized, placebo-controlled trial. *Alcohol Clin Exp Res.* 2015; 39(11): 2189–2199. <https://doi.org/10.1111/acer.12854> PMID: [26414337](https://pubmed.ncbi.nlm.nih.gov/26414337/)
25. Litten RZ, Ryan ML, Fertig JB, Falk DE, Johnson B, Dunn KE, et al. A double-blind, placebo-controlled trial assessing the efficacy of varenicline tartrate for alcohol dependence. *J Addict Med.* 2013; 7(4): 277–286. <https://doi.org/10.1097/ADM.0b013e31829623f4> PMID: [23728065](https://pubmed.ncbi.nlm.nih.gov/23728065/)
26. Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, et al. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology.* 2007; 52(3): 985–994. <https://doi.org/10.1016/j.neuropharm.2006.10.016> PMID: [17157884](https://pubmed.ncbi.nlm.nih.gov/17157884/)
27. Azam L, Winzer-Serhan UH, Chen Y, Leslie FM. Expression of neuronal nicotinic acetylcholine receptor subunit mRNAs within midbrain dopamine neurons. *J Comp Neurol.* 2002; 444(3): 260–274. <https://doi.org/10.1002/cne.10138> PMID: [11840479](https://pubmed.ncbi.nlm.nih.gov/11840479/)
28. Hieronymus F, Nilsson S, Eriksson E. A mega-analysis of fixed-dose trials reveals dose-dependency and a rapid onset of action for the antidepressant effect of three selective serotonin reuptake inhibitors. *Transl Psychiatry.* 2016; 6(6): e834. <https://doi.org/10.1038/tp.2016.104> PMID: [27271860](https://pubmed.ncbi.nlm.nih.gov/27271860/)
29. Cortese S, Adamo N, Del Giovane C, Mohr-Jensen C, Hayes AJ, Carucci S, et al. Comparative efficacy and tolerability of medications for attention-deficit hyperactivity disorder in children, adolescents, and adults: a systematic review and network meta-analysis. *Lancet Psychiatry.* 2018; 5(9): 727–738. [https://doi.org/10.1016/S2215-0366\(18\)30269-4](https://doi.org/10.1016/S2215-0366(18)30269-4) PMID: [30097390](https://pubmed.ncbi.nlm.nih.gov/30097390/)
30. Jorenby DE, Leischow SJ, Nides MA, Rennard SI, Johnston JA, Hughes AR, et al. A controlled trial of sustained-release bupropion, a nicotine patch, or both for smoking cessation. *N Engl J Med.* 1999; 340(9): 685–691. <https://doi.org/10.1056/NEJM199903043400903> PMID: [10053177](https://pubmed.ncbi.nlm.nih.gov/10053177/)
31. Soderpalm B, Danielsson K, de Bejczy A, Adermark L, Ericson M. Combined administration of varenicline and bupropion produces additive effects on accumbal dopamine and abolishes the alcohol deprivation effect in rats. *Addict Biol.* 2020 Sep; 25(5): e12807. <https://doi.org/10.1111/adb.12807> PMID: [31293045](https://pubmed.ncbi.nlm.nih.gov/31293045/)
32. Spanagel R, Holter SM. Pharmacological validation of a new animal model of alcoholism. *J Neural Transm.* 2000; 107(6): 669–680. <https://doi.org/10.1007/s007020070068> PMID: [10943907](https://pubmed.ncbi.nlm.nih.gov/10943907/)
33. Varga A, Hansson P, Johnson G, Alling C. Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. *Clin Chim Acta.* 2000; 299(1–2): 141–150. [https://doi.org/10.1016/S0009-8981\(00\)00291-6](https://doi.org/10.1016/S0009-8981(00)00291-6) PMID: [10900300](https://pubmed.ncbi.nlm.nih.gov/10900300/)

34. Hartmann S, Aradottir S, Graf M, Wiesbeck G, Lesch O, Ramskogler K, et al. Phosphatidylethanol as a sensitive and specific biomarker: comparison with gamma-glutamyl transpeptidase, mean corpuscular volume and carbohydrate-deficient transferrin. *Addict Biol.* 2007; 12(1): 81–84. <https://doi.org/10.1111/j.1369-1600.2006.00040.x> PMID: 17407500
35. Hashimoto E, Riederer PF, Hesselbrock VM, Hesselbrock MN, Mann K, Ukai W, et al. Consensus paper of the WFSBP task force on biological markers: biological markers for alcoholism. *World J Biol Psychiatry.* 2013; 14(8): 549–564. <https://doi.org/10.3109/15622975.2013.838302> PMID: 24236956
36. Hill-Kapturczak N, Dougherty DM, Roache JD, Karns-Wright TE, Javors MA. Differences in the synthesis and elimination of phosphatidylethanol 16:0/18:1 and 16:0/18:2 after acute doses of alcohol. *Alcohol Clin Exp Res.* 2018; 42(5): 851–860. <https://doi.org/10.1111/acer.13620> PMID: 29505133
37. Gnann H, Weinmann W, Thierauf A. Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. *Alcohol Clin Exp Res.* 2012; 36(9): 1507–1511. <https://doi.org/10.1111/j.1530-0277.2012.01768.x> PMID: 22458353
38. Wurst FM, Thon N, Aradottir S, Hartmann S, Wiesbeck GA, Lesch O, et al. Phosphatidylethanol: normalization during detoxification, gender aspects and correlation with other biomarkers and self-reports. *Addict Biol.* 2010; 15(1): 88–95. <https://doi.org/10.1111/j.1369-1600.2009.00185.x> PMID: 20002024
39. Kessler RC, Adler L, Ames M, Demler O, Faraone S, Hiripi E, et al. The World Health Organization Adult ADHD Self-Report Scale (ASRS): a short screening scale for use in the general population. *Psychol Med.* 2005; 35(2): 245–256. <https://doi.org/10.1017/s0033291704002892> PMID: 15841682
40. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. *J Stud Alcohol.* 1995; 56(4): 423–432. <https://doi.org/10.15288/jsa.1995.56.423> PMID: 7674678
41. Babor TF, H-B J, Saunders JB, Monteiro MG. AUDIT. The Alcohol Use Disorders Identification Test. Guidelines for use in primary care. Geneva: World Health Organisation; 2001. WHO/MSD/MSB/01.6a. 2001. <https://apps.who.int/iris/handle/10665/67205>
42. Berman AH, Bergman H, Palmstierna T, Schlyter F. Evaluation of the Drug Use Disorders Identification Test (DUDIT) in criminal justice and detoxification settings and in a Swedish population sample. *Eur Addict Res.* 2005; 11(1): 22–31. <https://doi.org/10.1159/000081413> PMID: 15608468
43. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry.* 1979; 134: 382–389. <https://doi.org/10.1192/bjpp.134.4.382> PMID: 444788
44. Li Z, Shi HS, Elis O, Yang ZY, Wang Y, Lui SSY, et al. The structural invariance of the Temporal Experience of Pleasure Scale across time and culture. *Psych J.* 2018; 7(2): 59–67. <https://doi.org/10.1002/pchj.207> PMID: 29431282
45. Sobell LC, Sobell MB. Timeline Follow-back: A technique for assessing self-reported alcohol consumption. In: Litten R. Z. & J. P. Allen editors. *Measuring Alcohol Consumption: Psychosocial and Biological Methods.* New Jersey: Humana Press; 1992: pp. 41–72.
46. Aitken RC. Measurement of feelings using visual analogue scales. *Proc R Soc Med.* 1969; 62(10): 989–993. PMID: 4899510
47. Heinz A, Siessmeier T, Wrase J, Buchholz HG, Grunder G, Kumakura Y, et al. Correlation of alcohol craving with striatal dopamine synthesis capacity and D2/3 receptor availability: a combined [18F] DOPA and [18F]DMFP PET study in detoxified alcoholic patients. *Am J Psychiatry.* 2005; 162(8): 1515–1520. <https://doi.org/10.1176/appi.ajp.162.8.1515> PMID: 16055774
48. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Jayne M, et al. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci.* 2007; 27(46): 12700–12706. <https://doi.org/10.1523/JNEUROSCI.3371-07.2007> PMID: 18003850
49. Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry.* 2016; 3(8): 760–773. [https://doi.org/10.1016/S2215-0366\(16\)00104-8](https://doi.org/10.1016/S2215-0366(16)00104-8) PMID: 27475769
50. Volkow ND, Wang GJ, Begleiter H, Porjesz B, Fowler JS, Telang F, et al. High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. *Arch Gen Psychiatry.* 2006; 63(9): 999–1008. <https://doi.org/10.1001/archpsyc.63.9.999> PMID: 16953002
51. Weiss F, Parsons LH, Schulteis G, Hyttia P, Lorang MT, Bloom FE, et al. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci.* 1996; 16(10): 3474–3485. <https://doi.org/10.1523/JNEUROSCI.16-10-03474.1996> PMID: 8627380
52. Feltmann K, Fredriksson I, Wirf M, Schilström B, Steensland P. The monoamine stabilizer (-)-OSU6162 counteracts downregulated dopamine output in the nucleus accumbens of long-term drinking Wistar rats. *Addict Biol.* 2016; 21(2): 438–449. <https://doi.org/10.1111/adb.12304> PMID: 26464265

53. Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, et al. Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. *Am J Psychiatry*. 2004; 161(10): 1783–1789. <https://doi.org/10.1176/appi.ajp.161.10.1783> PMID: [15465974](https://pubmed.ncbi.nlm.nih.gov/15465974/)
54. Aubin HJ, Karila L, Reynaud M. Pharmacotherapy for smoking cessation: present and future. *Curr Pharm Des*. 2011; 17(14): 1343–1350. <https://doi.org/10.2174/138161211796150837> PMID: [21524268](https://pubmed.ncbi.nlm.nih.gov/21524268/)